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REVIEW

Putting it all together: intrinsic and extrinsic mechanisms governing proteasome biogenesis

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BACKGROUND: The 26S proteasome is at the heart of the ubiquitin-proteasome system, which is the key cellular pathway for the regulated degradation of proteins and enforcement of protein quality control. The 26S proteasome is an unusually large and complicated protease comprising a 28-subunit core particle (CP) capped by one or two 19-subunit regulatory particles (RP). Multiple activities within the RP process incoming ubiquitinated substrates for eventual degradation by the barrel-shaped CP. The large size and elaborate architecture of the proteasome have made it an exceptional model for understanding mechanistic themes in macromolecular assembly.
OBJECTIVE: In the present work, we highlight the most recent mechanistic insights into proteasome assembly, with particular emphasis on intrinsic and extrinsic factors regulating proteasome biogenesis. We also describe new and exciting questions arising about how proteasome assembly is regulated and deregulated in normal and diseased cells.
METHODS: A comprehensive literature search using the PubMed search engine was performed, and key findings yielding mechanistic insight into proteasome assembly were included in this review.
RESULTS: Key recent studies have revealed that proteasome biogenesis is dependent upon intrinsic features of the subunits themselves as well as extrinsic factors, many of which function as dedicated chaperones.
CONCLUSION: Cells rely on a diverse set of mechanistic strategies to ensure the rapid, efficient, and faithful assembly of proteasomes from their cognate subunits. Importantly, physiological as well as pathological changes to proteasome assembly are emerging as exciting paradigms to alter protein degradation *in vivo*.

Keywords proteasome assembly, assembly chaperones, ubiquitin-proteasome system, proteolysis, macromolecular complex

Introduction

The ubiquitin-proteasome system (UPS) is the primary mechanism for regulatory and quality control protein degradation in eukaryotic cells (Finley, 2009; Tomko and Hochstrasser, 2013). Nearly every biological pathway depends in some way on protein degradation by the UPS to perform cellular functions, or to ensure the integrity of its components. Proteins destined for destruction by the UPS are typically modified via the covalent attachment of the small protein ubiquitin (Ub) to one or more lysine residues in the target protein. Additional Ub molecules can then be attached to lysines in the original Ub to form a polyubiquitin (pUb) chain. This pUb chain in turn serves as a signal for delivery to the 26S proteasome. The 26S proteasome is a large multisubunit ATP-dependent protease complex present in all eukaryotes, and represents the endpoint for proteins destined for degradation by the UPS. The exceptional complexity and large size of the proteasome has made it an excellent model for understanding how complicated macromolecular structures can be assembled rapidly and faithfully from dozens of components in cells. Recent groundbreaking advances in the structure of the proteasome (Lander et al., 2012; Lasker et al., 2012; Matyskiela et al., 2013; Sledz et al., 2013; Pathare et al., 2014; Unverdorben et al., 2014; Worden et al., 2014; Dambacher et al., 2016; Luan et al., 2016; Schweitzer et al., 2016) and the identification of dedicated assembly chaperones (Ramos et al., 1998; Hirano et al., 2005; Hirano et al., 2006; Le Tallec et al., 2007; Li et al., 2007; Kusmierczyk et al., 2008; Funakoshi et al., 2009; Kaneko et al., 2009; Le Tallec et al., 2009; Roelofs et al., 2009; Saeki et al., 2009) that facilitate proteasome biogenesis have yielded unprecedented insights into how nature manages the

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challenges of macromolecular assembly, and has revealed important parallels to assembly of numerous other multi-subunit complexes. In this review, we discuss the basic mechanisms of proteasome assembly, with an emphasis on how intrinsic features of subunits cooperate with extrinsic assembly chaperones to ensure efficient proteasome biogenesis *in vivo*. Finally, we comment on arising questions in our understanding of proteasome assembly in vivo, and their links to human disease.

Proteasome structure and function

In eukaryotes, the 26S proteasome consists of a barrel-shaped 20S core particle (CP) that houses interior protease sites, and a 19S regulatory particle (RP) that abuts one or both open ends of the CP (Fig. 1A). The CP consists of four axially stacked heteroheptameric rings. In eukaryotes, the outer rings are each composed of seven α-subunits (α1-α7; Fig. 1B), whereas seven β-subunits (β1-β7; Fig. 1C) comprise each inner ring. The three proteolytic activities of the proteasome, caspase-like, tryptic-like, and chymotryptic-like, are housed at the interface between the β rings and are encoded by the β1, β2, and β5 subunits (Arendt and Hochstrasser, 1997; Heinemeyer et al., 1997), respectively. These activities cooperate to cleave substrates into short peptides. The distinct specificities of these proteolytic sites ensure that substrates with diverse primary sequences can be processed efficiently by the proteasome. In mammals, four additional β subunits have been discovered: β1i, β2i, β5i, and β5t. These subunits replace the canonical catalytic β subunits within the CP, thus altering the proteolytic specificity of the CP and forming immunoproteasomes and thymoproteasomes. Immunoproteasomes enhance loading of peptides onto the class I major histocompatibility complex for immune presentation to killer T cells (Gaczynska et al., 1993; Kloetzel, 2004), whereas thymoproteasomes increase the repertoire of "self" peptides for positive selection during T cell development (Murata et al., 2007).

The RP mediates the binding of substrates, the removal of the pUb targeting signals (deubiquitination), and the unfolding and translocation of substrates into the CP for degradation. The RP can be further divided into lid and base subcomplexes. The lid consists of nine regulatory particle non-ATPase (Rpn) subunits (Rpn3, Rpn5-9, Rpn11, Rpn12, Sem1) (Fig. 1D). The base is composed of six regulatory particle, triphosphatase (Rpt) subunits, Rpt1-6, which are AAA + family ATPases that form a hexameric ring with a central pore, and four Rpn substrate receptor subunits, Rpn1, Rpn2, Rpn10, and Rpn13 (Fig. 1E). During proteolysis, the incoming substrate is captured via interaction of the pUb chain with one or more substrate receptor subunits, and the ATPase ring uses chemical energy derived from ATP hydrolysis to mechanically unfold the substrate and translocate it through the central pore of the ATPase ring. As the

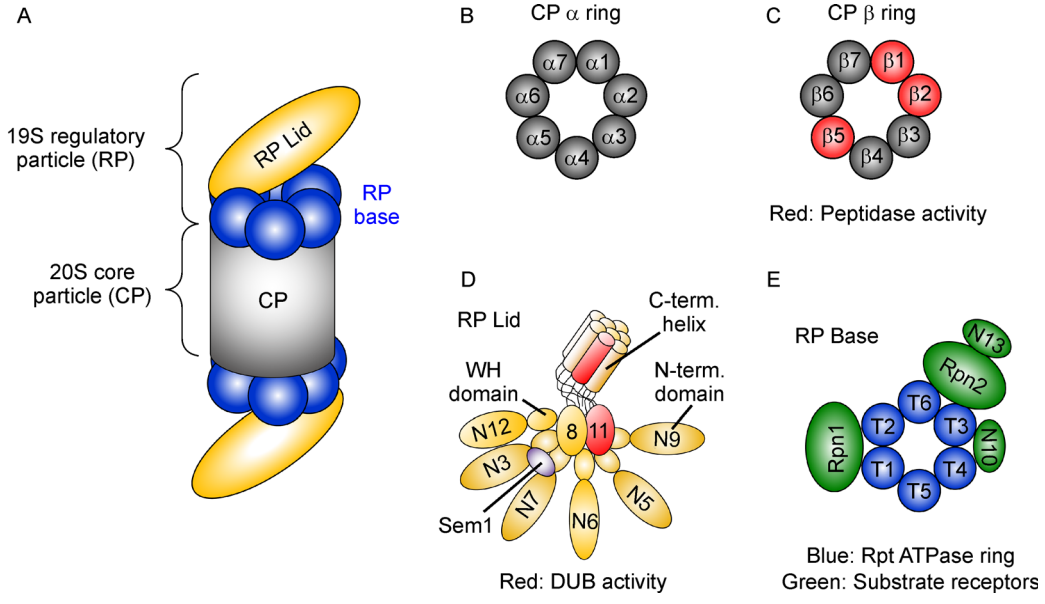

Figure 1 Architecture and composition of the proteasome. (A) The 26S proteasome consists of a 20S core particle (CP), shown in gray, capped on one or both ends by the 19S regulatory particle (RP). The RP can be further divided into lid and base subcomplexes, shown in yellow and blue, respectively. (B) Architecture of the CP α ring. (C) Architecture of the β ring. The three β subunits harboring peptidase activity are in red, whereas the noncatalytic β subunits are in light gray. (D) Subunit arrangement and domain architecture of the RP lid. The lid consists of non-ATPase subunits Rpn3, 5-9, 11, 12, and Rpn15/Sem1. Rpn11, shown in red, harbors the lone intrinsic deubiquitinating (DUB) activity within the proteasome. (E) Subunit composition and architecture of the RP base. The six Rpt ATPases are shown in blue, and the four non-ATPase subunits, Rpn1, 2, 10, and 13, are shown in green. Non-ATPase subunits are shown in their relative positions within the RP. Note that Rpn10 does not directly contact Rpt3 and Rpt4, but rather is suspended above them via subunits of the lid.

substrate is threaded through the pore of the ATPase ring, Rpn11 deubiquitinates the substrate so that ubiquitin can be recycled (Matyskiela et al., 2013; Sledz et al., 2013; Pathare et al., 2014; Worden et al., 2014).

Framework of CP assembly

In eukaryotes, CP assembly begins with the formation of an α-ring (Zwickl et al., 1994; Hirano et al., 2008), which functions as a platform upon which β subunits are incorporated (Frentzel et al., 1994; Nandi et al., 1997; Schmidtke et al., 1997) (Fig. 2). Entry of "early" β subunits β2, β3, β4 results in the formation of the 13S intermediate (Hirano et al., 2008). This is the smallest assembly intermediate that can be detected in yeast cells (Li et al., 2007). Subsequent entry of β5, β6, and β1 gives rise to the 15S intermediate (Hirano et al., 2008), alternatively referred to as the "–β7 half-mer" (Li et al., 2007). In both yeast and mammals, β7 is the last β subunit to incorporate (Marques et al., 2007; Hirano et al., 2008; Li et al., 2016) leading to a transient species called the half-proteasome. Two half-proteasomes dimerize to generate the preholoproteasome (PHP), which is a 20S complex with β propeptides still intact (Mayr et al., 1998b; Groll et al., 2003; Li et al., 2007). The processing of β subunit propeptides converts PHP to a fully active CP (Fig. 2). Activation of β subunits is concurrent with (or immediately follows) PHP formation; it involves both autocatalytic processing of propeptides and trimming of neighboring propeptides by activated β subunits (Chen and Hochstrasser, 1996; Schmidtke et al., 1996; Nandi et al., 1997).

Framework of RP assembly

Unlike the CP, which is comprised entirely of ring structures, the RP contains more heterogeneity in architecture. The base and lid subcomplexes can assemble independently of one another (Lander et al., 2012; Beckwith et al., 2013; Tomko and Hochstrasser 2014; Tomko et al., 2015). Most evidence indicates that these complexes normally complete assembly before assembling into the RP although some reports suggest alternative pathways may exist in which lid and base subunits associate prior to completion of their respective complexes (Thompson et al., 2009; Yu et al., 2015).

Lid biogenesis proceeds independently of the CP or base, and coexpression studies suggest that lid assembly initiates with the dimerization of Rpn8 and Rpn11, followed by recruitment of Rpn6 (Estrin et al., 2013) (Fig. 3A). Rpn6 then serves to recruit Rpn5 and Rpn9 to form a complex referred to as Module 1 (Sharon et al., 2006). In a parallel arm, Rpn3 and Rpn7 are brought together by Sem1 to form a heterotrimeric complex referred to as lid particle 3 (LP3) (Fukunaga et al., 2010; Tomko and Hochstrasser, 2011; Tomko and Hochstrasser, 2014). These two subcomplexes then associate to form a nearly complete lid intermediate lacking only Rpn12, called lid particle 2 (LP2) (Tomko and Hochstrasser, 2011). Rpn12 then associates to complete the biogenesis of the lid

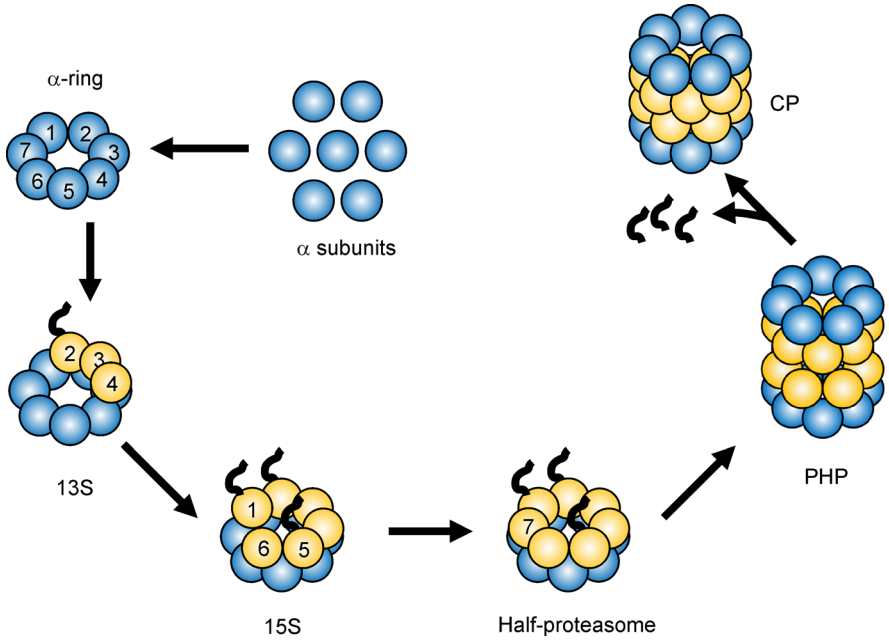

Figure 2 Framework of CP assembly. For clarity, assembly factors have been omitted and β subunit propeptides (squiggly lines) are shown only on the catalytically active subunits. CP assembly begins when α subunits coalesce into an α-ring. Early β subunits (β2, β3, β4) bind to the α-ring to form the 13S intermediate. Subsequent entry of the late β subunits (β5, β6, β1) results in the formation of the 15S intermediate. Incorporation of β7 is the rate limiting step of CP assembly and gives rise to a complete half-proteasome. Dimerization of two half-proteasomes forms a transient species, the preholoproteasome (PHP), which undergoes processing of the β subunit propeptides to form the mature CP.

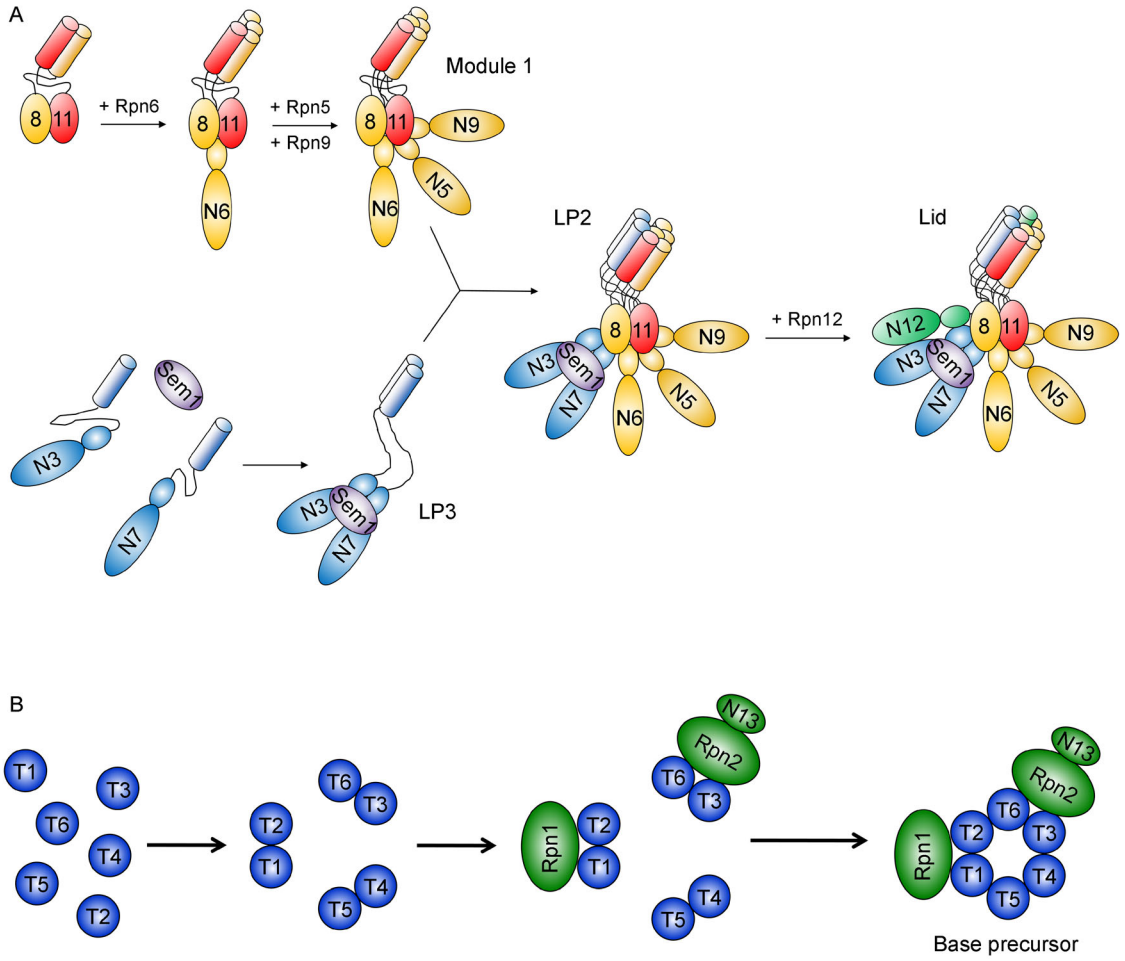

Figure 3 The lid and base assembly pathways. (A) Lid assembly pathway in yeast. (B) Overview of base assembly. Assembly chaperones are omitted for clarity and are addressed in Fig. 7.

(Fukunaga et al., 2010; Tomko and Hochstrasser, 2011; Tomko et al., 2015).

The foundation for the base is the heterohexameric ATPase ring, which contains the six Rpt subunits arranged in the order Rpt1-Rpt2-Rpt6-Rpt3-Rpt4-Rpt5 (Tomko et al., 2010). Each of these subunits shares a high degree of sequence and structural similarity with one another, analogous to the subunits of the CP. Formation of the base appears to initiate with the formation of three ATPase dimers (Fig. 3B). These dimers assemble in part via N-terminal coiled coil domains that facilitate pairing (Zhang et al., 2009). The ATPase dimers recruit the non-ATPase subunits Rpn1, Rpn2, and Rpn13, and the resultant intermediates assemble to form the nascent base subcomplex. The nascent lid and base are then proficient for association to form the RP.

Challenges in efficient proteasome assembly

Although the 26S proteasome consisting of RP and CP is unique to eukaryotes, the 20S CP is present in all eukaryotes, all archaea, and the actinomycete lineage of bacteria (Tomko and Hochstrasser, 2013). The 20S proteasomes of archaea and some bacteria are much less complicated than those of eukaryotes. In these organisms, proteasomes lack obvious substrate receptor and lid subunits, and instead are typically composed of only a single type of α and β subunit, which form homomeric rings. These homomeric CPs are generally capped by a single homomeric ATPase (Zwickl et al., 1999; Benaroudj and Goldberg, 2000; Barthelme and Sauer, 2012b; Forouzan et al., 2012). In the case of these more homogenous proteasomes, the assembly process is relatively simple. Briefly, bacterial α and β subunits associate to form heterodimers, which then associate laterally to form a homoheptameric α ring stacked on a homoheptameric β ring (Zuhl et al., 1997; Sharon et al., 2007). Two of these "half-proteasomes" can then associate to form a full CP (Mayr et al., 1998a; Kwon et al., 2004a), which can be subsequently capped by the hexameric ATPase ring. Archaeal α subunits form rings first (Zwickl et al., 1994), and these act as a template for β-ring assembly until a half-proteasome is

formed, though a bacterial-like assembly pathway is also possible (Panfair et al., 2015). In support of such simple, autonomous assembly, heterologous expression of α, β, and ATPase subunits typically results in formation of properly assembled, active proteasomes. In the case of these proteasomes, only a few logistical issues must be addressed to form functional particles. Specifically, rings must form from the proper number of subunits, and the stacking of subunits to form rings must occur in a manner that does not interfere with completion of each ring. The size of the ring is probably predetermined, because the curvature of the ring is controlled by the structure of the homomeric subunits that compose it. Also, whether a given ring forms completely prior to association with subunits of a neighboring ring is dictated by the relative affinities of the subunits within that ring for each other versus their affinities for subunits of the neighboring ring. Although the overall architecture of proteasomes is retained in eukaryotes, the composition is much more complex due to diversification of α, β, and ATPase subunits within the proteasome, as well as the presence of substrate receptors and the lid. Such diversification yields many additional challenges to efficient and faithful proteasome biogenesis. Subunit heterogeneity typically imposes specific positions for individual subunits within a given ring, and it necessitates that rings associate with a proper register to one another. As the seven α, seven β, and six ATPase subunits evolved via diversification from a common ancestral α, β, or ATPase subunit (Wollenberg and Swaffield, 2001; Gille et al., 2003), they share substantial sequence and structural similarity with their orthologs, and are in some cases prone to misassembly (Gerards et al., 1997; Gerards et al., 1998; Yao et al., 1999; Takeuchi and Tamura 2004; Ishii et al., 2015). Thus, additional control mechanisms are necessary to limit the formation of products that are nonproductive for proteasome biogenesis and could potentially even be toxic. Similarly, formation of assembly intermediates that sterically occlude or otherwise interfere with incorporation of a complete set of subunits must be avoided. Finally, as proper enzymatic coupling of substrate binding, deubiquitination, unfolding, and proteolysis is necessary for proper function, the activities of the eukaryotic lid, base, and CP must be suppressed until the proteasome has fully assembled.

Despite these challenges, proteasome biogenesis occurs very rapidly and with near-perfect fidelity in normal cells. A substantial number of evolutionarily conserved regulatory mechanisms, mediated both by intrinsic subunit features and extrinsic assembly chaperones, cooperate to ensure such fast and faithful assembly *in vivo*. These mechanisms function to sculpt and guide the formation of a limited number of assembly intermediates that, in many cases, then associate via defined, hierarchical assembly pathways to yield mature, functional proteasomes. We review the best understood examples of these intrinsic and extrinsic regulatory mechanisms herein focusing on the canonical eukaryotic 26S proteasome.

Proteasome assembly chaperones and their mechanisms of action

In this section, we discuss what is known about the role of assembly chaperones in the biogenesis of the proteasome, starting with the CP. Where appropriate, both yeast and mammalian terminology will be used. However, when referring to assembly in general, yeast terminology will be used to streamline the discussion.

CP chaperones

Five conserved eukaryotic proteins comprise three confirmed dedicated assembly chaperones in CP biogenesis (Pba1-Pba2; Pba3-Pba4; Ump1). A sixth protein (Blm10) may also play a role in CP assembly. In general, the assembly chaperones fulfill both positive functions (i.e. actively promote desired assembly events) and negative functions (i.e. prevent undesired assembly events) during CP formation.

Pba1-Pba2/PAC1-PAC2

This heterodimeric chaperone is involved at all stages of CP assembly and likely fulfils several roles. The easiest of these to visualize is that of a "safety" that prevents premature association of RP with CP (Fig. 4A) (Kusmierczyk et al., 2011; Wani et al., 2015). The binding of RP to CP is mediated in part by highly conserved C-terminal HbYX motifs (Hb = hydrophobic; Y = tyrosine (or phenylalanine); X = any amino acid) present on select Rpt subunits of the base (Smith et al., 2007; Gillette et al., 2008; Yu et al., 2010). These motifs insert into pockets formed by two adjacent α subunits, one of which contributes a conserved lysine that forms a salt bridge with the C-terminal carboxylate of the HbYX motif (Tian et al., 2011; Park et al., 2013) (Fig. 5 and also see below). Thus, RP has the potential to interact with any species containing these pockets, including mature CP, as well as any CP intermediates with a full (or even potentially an incomplete) α-ring. Pba1 and Pba2 also contain functional HbYX motifs (Kusmierczyk et al., 2011) which allow them to interact with the same α-ring surface as RP (Stadtmueller et al., 2012). The HbYX motif of Pba1 can insert into the pocket at the interface between α5 and α6 (forming a salt-bridge with the α6 pocket lysine) whereas that of Pba2 inserts into the pocket between α6 and α7. In mature 26S proteasomes, the HbYX motif of Rpt5 inserts into the same pocket as used by Pba1 (Beck et al., 2012; Schweitzer et al., 2016) though it can also occupy the pocket used by Pba2 (Tian et al., 2011). However, Pba1-Pba2 has much higher affinity for α-rings present within CP intermediates than within those of mature CP, whereas the opposite is true for RP (Wani et al., 2015). This explains why Pba1-Pba2 is able to function as a safety in preventing inappropriate RP interaction with immature CP. Once mature CP has formed, the higher affinity of RP for CP effectively outcompetes Pba1-Pba2 for the α-ring surface.

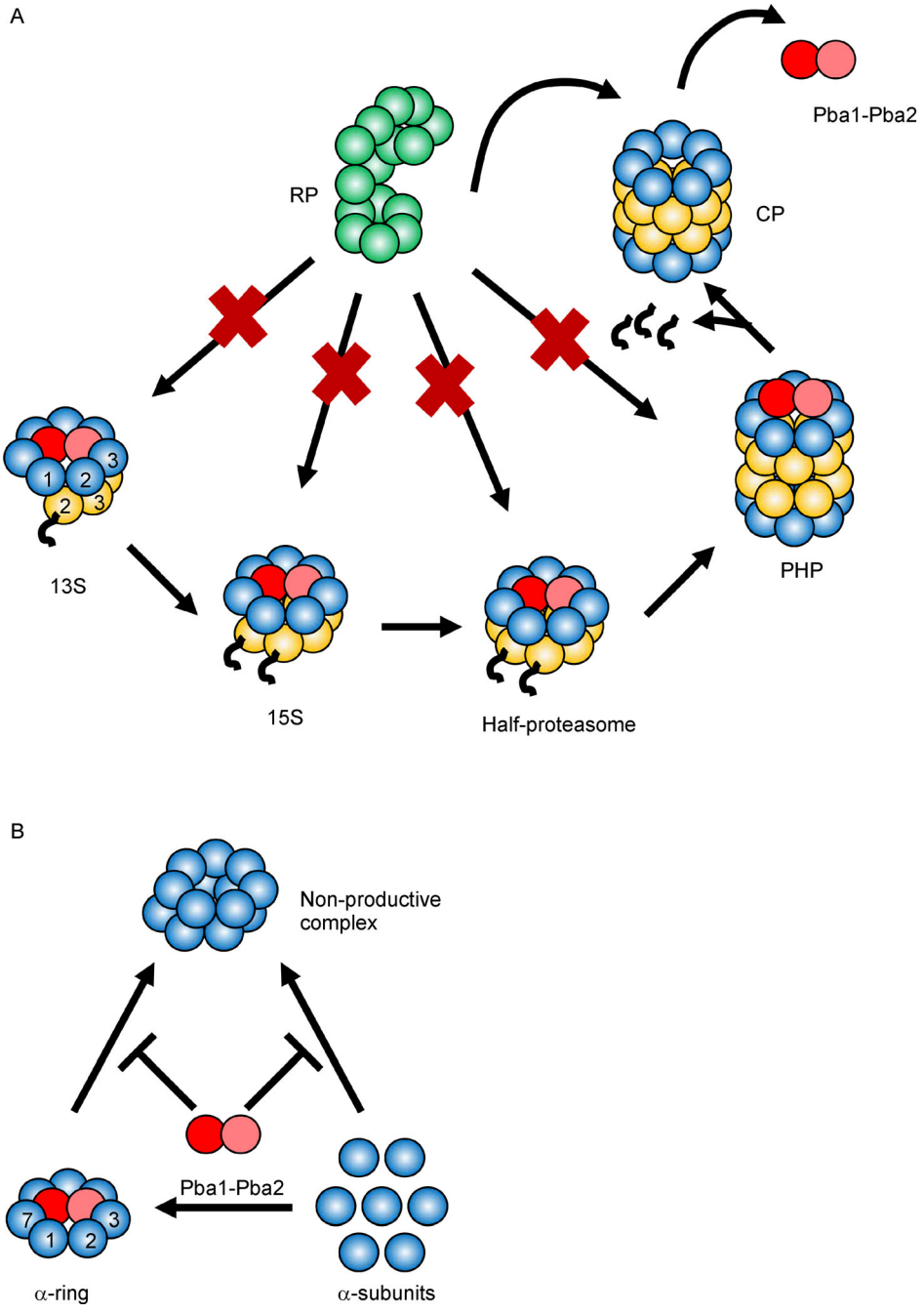

Figure 4 Pba1-Pba2 and CP assembly. (A) Pba1-Pba2 functions as a safety. Pba1-Pba2 is shown bound to a series of CP assembly intermediates containing a complete α-ring and various β subunits. The intermediates are shown inverted, relative to their orientation in Fig. 2, to better visualize the binding of the assembly factor. The α-ring in the intermediates prior to the preholoproteasome (PHP) stage is distended, which allows Pba1-Pba2 to lie partially embedded in the axial channel formed by the ring. This is the high affinity state of Pba1-Pba2 bound to an α-ring and makes it impossible for RP to occupy the ring. Following dimerization of half-proteasomes, each α-ring undergoes a conformational change which tightens its radius as the α subunits move closer to the central axis. The resulting narrowing of the axial channel evicts Pba1-Pba2 which assumes a more surface-bound location. As the propeptides are processed and a mature CP forms, Pba1-Pba2 binding switches to a low affinity state which allows it to be easily displaced from the now-functional CP by the RP. (B) Additional functions of Pba1-Pba2. The formation of α-rings is promoted, in an unknown fashion, by Pba1-Pba2. At the same time, Pba1-Pba2 binding to α subunits and/or isolated α-rings prevents these entities from misassembly into non-productive species.

It is not yet clear what causes this affinity switch of Pba1-Pba2 for the α-ring. *In vitro* studies of the archaeal ortholog of Pba1-Pba2 suggest that the processing status of β subunit propeptides may be transmitted to the outer surface of the α-ring (Kusmierczyk et al., 2011). Arguing in favor of the existence of such long distance communication are observations that RP binding to α-rings can be affected by occupancy of β subunit active sites (Kleijnen et al., 2007) and that pocket

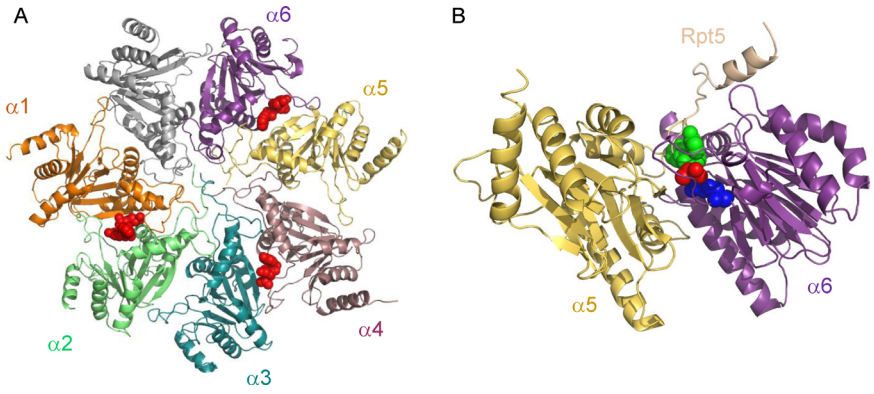

Figure 5 HbYX motif docking into the CP α-ring. (A) A view of the Rpt2, Rpt3, and Rpt5 HbYX motifs docked into the intra-subunit pockets on the outside surface of the CP α ring. The HbYX motifs of Rpt2, Rpt3, and Rpt5 are shown as red spheres, and lie at the interfaces between α1-α2, α3-α4, and α5-α6, respectively. (B) A close-up view of the HbYX motif of Rpt5 docked into the α5-α6 pocket. Rpt5 (beige) inserts its three most C-terminal residues, Phe-Tyr-Ala, into the pocket. The C-terminal carboxylate of the alanine residue (red spheres) interacts with the positively charged side chain of the pocket lysine (blue) contributed by α6, whereas the Phe and Tyr residues (green spheres) make hydrophobic contacts with the interior of the pocket.

lysine mutations result in defects in the processing of β subunit propeptides (Park et al., 2011). However, this cannot be the only contributing factor to the affinity switch, as there are CP intermediates which contain a complete α-ring yet little-to-no β subunits, such as the 13S intermediate or the α-ring proper. And these too must be restricted from binding RP prematurely. Thus, alterations within α-rings themselves, or with their interactions with the HbYX motifs of Pba proteins, must also contribute. In support of this, recent evidence demonstrates that α-rings in the 15S intermediate are much broader than in mature CP (Kock et al., 2015). This allows Pba1-Pba2 to sit, partly embedded, in the enlarged pore of the α-ring where it makes contact with most of the α subunits. Increased contacts with the α-ring could contribute to increased affinity for precursor species. By contrast, Pba1-Pba2 has limited contacts with the α-ring in mature CP, mediated primarily via the HbYX motifs (Stadtmueller et al., 2012). A broadened α-ring would also perturb the conformation of the inter-α-subunit pockets, which should alter the HbYX-based interaction with them (Kock et al., 2015; Wani et al., 2015). Consistent with this is the observation that deletion of both HbYX motifs is required to abolish Pba1-Pba2 interaction with immature CP species, whereas deletion of only the Pba1 HbYX motif is sufficient to abrogate Pba1-Pba2 interaction with mature CP (Stadtmueller et al., 2012; Wani et al., 2015). As the α-ring contracts during the transition from 15S to the PHP, Pba1-Pba2 is squeezed out of its embedded position and adopts the location observed in the crystal structure (Stadtmueller et al., 2012; Kock et al., 2015) where it is subsequently displaced by RP (Fig. 4A).

Another function ascribed to Pba1-Pba2 is that of a factor that promotes (or stabilizes) the formation of α-rings from individual α subunits (Fig. 4B). This is based on two types of evidence. First, knockdown of mammalian PAC1-PAC2 results in decreased α-ring formation (Hirano et al., 2005) whereas in yeast cells lacking Pba1-Pba2, immature CP species containing structurally unstable α-rings, from which α5 and α6 readily dissociate, can be isolated (Wani et al., 2015). Second, both PAC1-PAC2 (Hirano et al., 2005) and Pba1-Pba2 (Le Tallec et al., 2007; Kusmierczyk and Hochstrasser, 2008) can associate with subsets of α subunits in vitro and in vivo, consistent with these being intermediates of α-ring assembly. PAC1-PAC2 can also bind to individual α subunits *in vitro* (Hirano et al., 2005), arguing that this assembly factor may function from the very beginning of CP assembly. Since isolated α subunits cannot form a pocket to recognize a HbYX motif, this indicates that PAC1-PAC2 is capable of non-HbYX-mediated binding in addition to HbYX-mediated binding. Taken together, these findings support a role in α-ring assembly (or stabilization), even if the precise mechanism remains to be determined. The ability of the embedded Pba1-Pba2 within isolated 15S species to contact most of the α subunits, via both HbYX-dependent and HbYX-independent interactions, suggests a simple model (Fig. 4B). Assuming the manner of Pba1-Pba2 binding to 15S intermediates is indicative of its binding to isolated α-rings, this assembly factor serves as a scaffold upon which α-rings are built (Hirano et al., 2005; Kock et al., 2015; Wani et al., 2015).

Knockdown of mammalian PAC1-PAC2 not only decreases α-ring formation, but also shifts the population of α subunits into larger species that do not contain β subunits or components of the RP (Hirano et al., 2005). Since certain individual eukaryotic α subunits produced recombinantly in bacteria readily assemble into double rings (Gerards et al., 1997; Ishii et al., 2015), and can even recruit their immediate α-ring neighbors into these structures (Gerards et al., 1998), the simple interpretation is that these larger species are α-ring dimers. Thus, the third function proposed for Pba1-Pba2 has been a role in preventing the formation of α-ring dimers (Fig. 4B). However, this role is probably not due to Pba1-Pba2 directly preventing two complete α-rings from coming

together. Double α-rings interact via a saw-toothed interface mediated primarily by H1 helices (Panfair et al., 2015) akin to the interaction between α- and β-rings in the half proteasome (Zwickl et al., 1994). However, Pba1-Pba2 binds to the opposite (i.e. outer) surface of the α-ring, where it would not produce steric interference to ring dimerization. Perhaps Pba1-Pba2 binding results in an α-ring conformation that is not capable of dimerizing; the distended α-ring found in the 15S could be such a species (Kock et al., 2015). Or, perhaps Pba1-Pba2 binding alters the order of subunit association; this could prevent pathways that lead to α-ring dimers (or any non-productive complex, for that matter) from becoming populated. We favor this latter possibility precisely because it does not limit the identity of non-productive complexes, which can form in the absence of Pba1-Pba2, to α-ring dimers only.

Pba3-Pba4/PAC3-PAC4

This heterodimer functions early in assembly and is associated with CP intermediates up until the 13S stage (Fig. 6) (Hirano et al., 2006; Le Tallec et al., 2007; Hirano et al., 2008; Yashiroda et al., 2008). Its early exit from the assembly pathway is explained by the manner in which it interacts with a nascent CP. *In vitro* Pba3-Pba4 binds tightly to α5 (Yashiroda et al., 2008) and subcomplexes of α subunits that contain α5 (Kusmierczyk et al., 2008). The binding of Pba3-Pba4 to helices H1 and H2 of α5, located on the α subunit surface that faces β subunits, is not compatible with the presence of β subunits, and it is displaced from the ring by incoming β4 (Hirano et al., 2008). Pba3-Pba4 has a unique function among the assembly chaperones in that it ensures the formation of canonical 20S proteasomes in which each subunit is represented in its "proper" place (Kusmierczyk et al., 2008). The α3 subunit is not essential in *Saccharomyces cerevisiae* and in its absence yeast synthesize an alternative proteasome in which a second copy of α4 takes the place of the missing α3 (Velichutina et al., 2004). These "α4–α4 proteasomes" also form in yeast when Pba3-Pba4 is absent, despite the continuing presence of α3 (Kusmierczyk et al., 2008). This argues that the efficient formation of normal α-rings requires Pba3-Pba4 function (Fig. 6).

As with Pba1-Pba2, the precise mechanism by which Pba3-Pba4 contributes to α-ring formation is not known. The key to understanding how Pba3-Pba4 functions begins with determining the significance of α4–α4 proteasome formation. Evidence for the physiologic relevance of α4–α4 proteasomes takes several forms. First, the non-essential nature of α3 extends to other fungal species including *Schizosaccharomyces pombe* (Kim et al., 2010a), *Neurospora crassa* (Colot et al., 2006), and probably *Aspergillus nidulans,* where an identified α3 allele is likely to be assembly incompetent (Lee and Shaw, 2007). This argues that dispensability of α3 is not a quirk of *S. cerevisia*e genetics. Second, the ability to form α4–α4 proteasomes has been demonstrated recently in mammalian cells, and the levels of these proteasomes correlate inversely with levels of PAC3 in the cell, echoing observations in yeast (Padmanabhan et al., 2016). Third, the ability to form α4–α4 proteasomes correlates with resistance to certain heavy metal stresses, a phenotype that is conserved from yeast to humans (Kusmierczyk et al., 2008; Padmanabhan et al., 2016). Fourth, α4–α4 proteasome levels can be modulated by altered levels of known oncogenes and tumor suppressors, a condition representative of a number of malignancies (Padmanabhan et al., 2016). The conserved ability of α subunits to assemble into canonical and α4–α4 proteasomes, both of which are physiologically relevant, requires two types of α-ring to form. This implies that the pathway(s) to α-ring formation must diverge at some point to allow this to occur. Recently, a non-canonical complex was isolated from yeast cells lacking Pba3-Pba4 (Takagi et al., 2014). It contained β2, β3, β4, and all α subunits except α4. Notably, α2 was present in twofold excess. This complex has been proposed to resemble a 13S intermediate in which α2 has taken the place of α4 in the α-ring (Fig. 6) (Takagi et al., 2014). Although this is likely a dead-end complex, if this complex does contain an α-ring with a third arrangement of subunits, this supports a model in which formation of α rings diverges subsequent to formation of an α1, α5, α6, α7 heterotetramer (Fig. 6).

The three rings formed in the absence of Pba3-Pba4 each contain α5, α6, α7, and α1 in their proper place, whereas α2, α3, and α4 can associate in a non-canonical manner. Thus, one can propose that one half of the ring (containing the contiguous subunits α5 through α1) assembles the same regardless of Pba3-Pba4 status. In analogy to β-ring formation, perhaps these are the "early" α subunits of α-ring assembly. The α2, α3, α4 subunits can complete the ring in several combinations, two of which are competent for further assembly, but the activity of Pba3-Pba4 ensures the canonical placement of α2, α3, and α4 and promotes the formation of the canonical CP. In wild-type yeast, Pba3-Pba4 activity is sufficient to ensure that this is essentially the only type of CP formed. In mammalian cells, PAC3-PAC4 levels may not be sufficient to result solely in canonical CP (Padmanabhan et al., 2016).

How the canonical placement of α2, α3, and α4 is favored is not known, but when the co-crystal structure of Pba3-Pba4 with α5 was modeled onto to the CP structure, it was projected to make substantial contacts with α4 and α6 (Yashiroda et al., 2008). This observation, in combination with the formation of the aberrant 13S-like complex lacking α4 when Pba3-Pba4 is absent in yeast, suggests that Pba3-Pba4 promotes the interaction of α5 with α4 (Takagi et al., 2014). Others have shown little retention of α4 on an affinity column in the presence of Pba3-Pba4 and α5 (Kusmierczyk et al., 2008), so it remains unclear if promoting α5-α4 interaction is the sole mechanism by which Pba3-Pba4 functions. Nevertheless, as with Pba1-Pba2, the presence of the Pba3-Pba4 assembly factor likely alters the order of association for some of the α subunits, favoring the formation of the canonical α-ring over others.

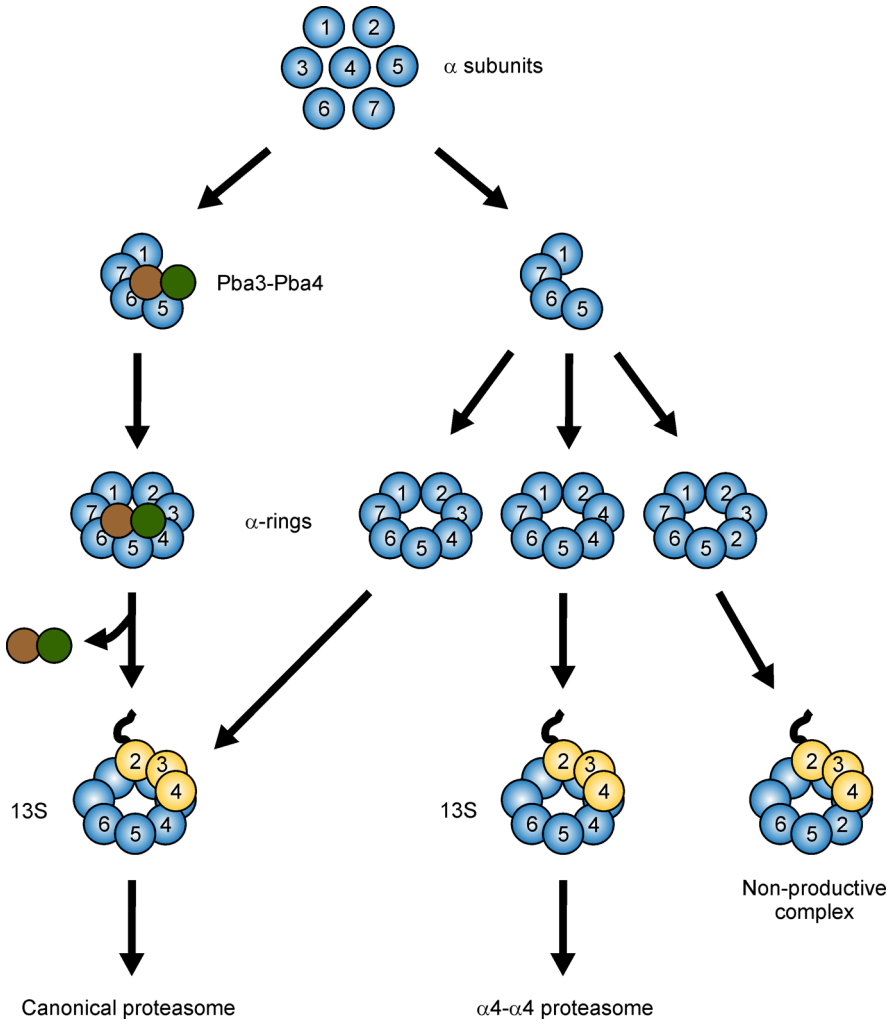

Figure 6 Pba3-Pba4 and α-ring assembly. The formation of α-rings is shown in the presence, or absence, of Pba3-Pba4. In both cases, the early events of α-ring assembly are similar and may involve the formation of species containing α5, α6, α7 and α1. However, via a poorly understood mechanism, the presence of Pba3-Pba4 influences the entry of the remaining α subunits (α2, α3 and α4) in an order that generates only canonical α-rings and thus only canonical proteasomes. Arrival of early β subunits displaces Pba3-Pba4, forming the 13S intermediate. In the absence of Pba3-Pba4, the remaining α subunits are not restricted in their order of assembly and (at least) three possible α-rings are formed. One of these is the canonical α-ring which is bound by β subunits to produce canonical proteasomes. Another is an α-ring in which α3 is replaced by a second copy of α4; this gives rise to α4-α4 proteasomes. The third ring, which lacks α4 and has two copies of α2, might form a 13S-like species that is not competent for further assembly.

Ump1/hUMP1/POMP/Proteassemblin

Although it was the first assembly factor to be discovered (Ramos et al., 1998), Ump1 has been the most recalcitrant to detailed biochemical and structural analysis – likely due to its intrinsically disordered state (Sa-Moura et al., 2013; Uekusa et al., 2014). Ump1 associates with CP precursors containing unprocessed β subunits, beginning with the 13S intermediate in yeast and a complete α-ring plus β2 in humans. It remains bound through half-proteasome dimerization and β subunit processing, and becomes encapsulated inside the CP and degraded upon completion of assembly (Frentzel et al., 1994; Ramos et al., 1998; Burri et al., 2000; Griffin et al., 2000; Witt et al., 2000; Li et al., 2007; Hirano et al., 2008). Yeast cells lacking Ump1 accumulate CP precursors, arguing for a positive role in assembly (Ramos et al., 1998); in human cells, this may entail actively promoting incorporation of β subunits such as β2 (Hirano et al., 2008) and β5i (Heink et al., 2005). However, genetic results have also suggested a negative role in assembly, specifically the prevention of premature dimerization of Ump1-containing precursors until a complete half-proteasome is formed (Li et al., 2007). Recent electron microscopy (EM) data combined with cross-linking and mass spectrometry (CX-MS) suggests a possible way to reconcile these two roles given Ump1's disordered nature (Kock et al., 2015). It posits Ump1 is splayed out along the interior of the 15S intermediate cavity, contacting a number of α and β subunits. Consistent with previous studies, it is likely the C-terminal two-thirds of this 16 kDa protein that contributes

these important binding contacts (Burri et al., 2000). Thus the C terminus might facilitate the incorporation/stabilization of β subunits. By contrast, the N-terminal third of Ump1, which is dispensable for CP binding (Burri et al., 2000), performs a checkpoint function. With CX-MS data placing it near β6, and potentially protruding out of the β-ring, the N terminus of Ump1 could be ideally positioned to both block dimerization and sense the arrival of β7 (Kock et al., 2015). If this is confirmed, it would explain how Ump1 delays dimerization until β7 incorporates, subsequently reorganizing and assuming a different orientation within the cavity, due to its disordered nature.

Blm10/PA200

Yeast Blm10 and mammalian PA200 are known proteasome activators, capable of binding CP alone or as a hybrid with RP (Ustrell et al., 2002; Schmidt et al., 2005). This HEAT-repeat protein forms a dome on the CP with an opening large enough to fit unfolded substrates and/or peptides (Sadre-Bazzaz et al., 2010; Dange et al., 2011). Blm10 has been demonstrated to promote CP import into the nucleus (Weberruss et al., 2013). However, it also appears to function during CP assembly (Fehlker et al., 2003). Blm10 associates with yeast 13S, 15S, and PHP intermediates (Li et al., 2007), presumably via its HbYX motif (Sadre-Bazzaz et al., 2010). Moreover, when deletion of the β7 tail is combined with a deletion of the *BLM10* gene, yeast exhibit a severe CP assembly defect (Marques et al., 2007). However, a precise function for Blm10 in CP assembly remains to be elucidated.

RP chaperones

As is the case for the subunits of the eukaryotic CP, the ATPase subunits do not encode all of the information required for their proper assembly in their primary sequences. Thus, the base also depends heavily upon extrinsic, dedicated assembly chaperones for proper formation. In yeast through humans, four dedicated RP-assembly chaperones, Nas2/p27, Hsm3/S5b, Nas6/p27, and Rpn14/PAAF1 (yeast/human) aid in the organization and temporal ordering of base subcomplex assembly (Funakoshi et al., 2009; Kaneko et al., 2009; Le Tallec et al., 2009; Park et al., 2009; Roelofs et al., 2009; Saeki et al., 2009). Although these chaperones are structurally dissimilar, they each bind to the C-terminal domain of a distinct Rpt subunit to form precursor assembly modules. These modules, Nas2-Rpt4–Rpt5, Hsm3-Rpt1-Rpt2-Rpn1, and Nas6-Rpt3–Rpt6-Rpn14-Rpn12-Rpn13 (hereafter called the Nas2, Hsm3, and Rpn14/Nas6 modules), then assemble sequentially to form the full base subcomplex. Although the precise mechanisms of the chaperones are still being elucidated, their major functions appear to be to regulate the association of the modules with one another, and to control the association of the base and its assembly intermediates with the CP. In some cases, denoted below, they also assist with the formation of the module itself by acting as scaffolds for incoming subunits.

Nas2/p27

Nas2 (p27 in humans) was initially described in mammalian cells as a subunit of the "modulator" complex, which was a trimeric complex reported to stimulate the peptidase activity of the CP (DeMartino et al., 1996). This complex contains p27, Rpt4, and Rpt5 subunits, and is now known to be the mammalian equivalent of the Nas2 assembly module of the base. Nas2 consists of an N-terminal helical domain and a C-terminal PDZ domain. Both of these domains bind to the Rpt5 C-terminal small domain of the AAA + ATPase fold (Lee et al., 2011; Satoh et al., 2014), but these interactions control assembly in different ways. PDZ domains typically recognize the extreme C-termini of their binding partners, including the free carboxylate. As such, Nas2 associates with the Rpt4-Rpt5 dimer in part via recognition of the three most C-terminal residues of Rpt5 (Lee et al., 2011). Importantly, these three residues constitute the Rpt5 HbYX motif, so binding of this tail by Nas2 precludes docking of the Rpt5 C terminus into the α-ring. In this way, Nas2 serves to regulate association of this module with the CP. The N-terminal helical domain of Nas2 binds the surface of the Rpt5 small domain that interacts with Rpt1 in the full base (Satoh et al., 2014). Nas2 must therefore be released from this surface before the Hsm3 module can stably integrate, and as such may serve to exclude the Hsm3 module from early base assembly intermediates. Indeed, Nas2 efficiently copurifies components of the Rpn14/Nas6 module, but fails to copurify any subunits of the Hsm3 module, consistent with this proposed mechanism (Tomko et al., 2010). Thus, Nas2 serves to regulate two distinct aspects of base assembly via a bivalent binding mechanism to a single protein domain. At the moment, the signal to eject Nas2 from assembling base intermediates is unknown, but it may result from association of the intermediate with the CP, or from conformational changes in its ATPase binding partner resulting from binding or hydrolysis of ATP.

Rpn14/PAAF1 and Nas6/gankyrin

The Rpn14/Nas6 (PAAF1/gankyrin in humans) module is unique in that it contains two chaperones that each recognize a distinct ATPase subunit within the module. Of the three modules, the functions of Rpn14 and Nas6 are probably the least characterized, although structures and general modes of binding to their cognate ATPases are known for both. Nas6 forms a long, curved structure characteristic of ankyrin repeat-containing proteins (Nakamura et al., 2007). A crystal structure of Nas6 in complex with its binding partner indicates that Nas6 uses the concave surface of this ankyrin fold to cradle the small, C-terminal domain of the Rpt3 AAA + fold, burying substantial surface area. In contrast, Rpn14 utilizes a cylindrical β-propeller structure composed of seven

WD40 repeats to associate with the C-terminal domain of Rpt6 (Kim et al., 2010b). Although the structure of Rpn14 is known, its binding interaction with Rpt6 has not been characterized at the atomic level. However, mutagenesis studies support a binding mode that is similar to that of Nas6, in which the top of the cylindrical β-propeller makes critical contacts with the C-terminal domain of Rpt6 (Kim et al., 2010b).

How binding of Nas6 and Rpn14 to Rpt3 and Rpt6, respectively, facilitates proteasome assembly is largely unknown. In yeast, the existence of the Rpn14/Nas6 module has been inferred solely on the basis of bimolecular interactions and coimmunoprecipitation experiments, confounding detailed architectural and mechanistic analysis. Thus, it remains unclear whether and exactly how the Rpn14/Nas6 module forms, and it is unknown if the chaperones facilitate pairing of Rpt3 and Rpt6 or the association of Rpn2 and Rpn13. However, modeling of Nas6 onto the ATPase ring of the proteasome suggests that it would clash sterically with the CP (Roelofs et al., 2009), at least under some conditions, and the same is likely true for Rpn14. Thus, these chaperones may restrict premature docking of this module onto the CP (Sokolova et al., 2015). A second possibility that is not yet explored, is that one or more of these subunits may serve to stabilize Rpn2 and Rpn13 in the context of the assembly intermediate. In recent structures of the 26S proteasome, Rpn13 contacts only Rpn2, and Rpn2 contacts the base only via the very N-termini of Rpt3 and Rpt6 (da Fonseca et al., 2012; Lander et al., 2012; Lasker et al., 2012). Instead, Rpn2-Rpn13 depends almost entirely on contacts with lid subunits for stabilization within the RP. As it is believed that the lid and base form separately, these critical stabilizing contacts would be absent in the assembling base; Rpn2 and Rpn13 may thus depend upon contacts with Rpn14 and/or Nas6 to stably associate with Rpt3 and Rpt6 during base biogenesis.

Hsm3/S5b

Hsm3 (S5b in humans) functions as both a chaperone and a scaffolding protein for the Hsm3 module. The protein sequence of Hsm3 consists primarily of ARM/HEAT repeats, and the protein forms a concave fold that cradles the C-terminal domain of Rpt1 (Barrault et al., 2012; Takagi et al., 2012; Park et al., 2013). The structure of Hsm3 in complex with the C-terminal domain of Rpt1 is highly reminiscent of the Nas6-Rpt6 C-terminal domain structure, in that it buries substantial surface of its concave face in Rpt1. This results in tight interaction with Rpt1, but in the context of the module, it also positions the chaperone to make stabilizing contacts with both Rpt2 and Rpn1 (Barrault et al., 2012). Hsm3 thus has a direct interaction with every subunit of this module. Mutations to any of these bridging contacts disrupts the formation of proteasomes *in vivo*, providing evidence that Hsm3 functions much as an assembly hub to recruit and stabilize each component of the complex (Barrault et al., 2012). Similar to that observed for Nas6, modeling of the Hsm3-Rpt1 C-terminal domain structure onto the full proteasome indicates that it would clash substantially with the CP (Park et al., 2013), suggesting that Hsm3 also serves to control the association of this module with the CP.

Adc17

Recently, an additional chaperone, Adc17, has been identified as a stress-inducible regulator of the Rpn14/Nas6 module in budding yeast. Adc17 was identified as a high-copy suppressor of lethality in response to heat stress in *cim3-1* mutant yeast, which harbor a missense mutation in the *RPT6* coding sequence (Hanssum et al., 2014). Adc17 associates with the N-terminal domain of Rpt6 and appears to promote Rpt3-Rpt6 dimerization, which in turn enhances proteasome assembly to maintain protein homeostasis. When proteasome activity becomes limiting, expression of new proteasome subunits is upregulated coincident with increased expression of Adc17. Upregulation of the Rpt6 subunit in particular appears to be dependent on Adc17, as deletion of Adc17 reduced protein levels of Rpt6. It is currently unclear why and how this particular function becomes necessary in response to stress, but it may be required to limit an inherent tendency of Rpt3 and/or Rpt6 to mispair or misfold under conditions of elevated expression. Many questions remain regarding this newly discovered chaperone, including how, if at all, it influences interaction between proteasomal subcomplexes, as well as how it is released from the nascent proteasome. No ortholog of Adc17 has yet been identified in metazoans (Hanssum et al., 2014), raising the intriguing possibility of organism-specific assembly chaperones.

Chaperone-dependent assembly of base precursor modules

Once precursor assembly modules are formed, Nas2, Nas6, Hsm3, and Rpn14 coordinate the stepwise assembly of the base subcomplex. There are currently two routes of chaperone-mediated base assembly that have been proposed. In the first, the base assembles from the three precursor modules *en vacuo* (Fig. 7A), whereas in the second, assembly of the base is templated by the CP (Fig. 7B). It is important to note that these two proposed pathways are not necessarily mutually exclusive. In both models, the association of modules occurs in an ordered fashion, although the exact order may differ between yeast and humans (Kaneko et al., 2009; Tomko et al., 2010), and in both cases, ejection of the chaperones is coupled to docking of the base (or a given base module) onto the CP.

Evidence for a template-independent model derives from initial observations that chaperone-bound base subcomplex is readily detectable in normal yeast (Funakoshi et al., 2009; Saeki et al., 2009). The observation that the full base contains chaperones but not CP, coupled with the absence of these chaperones in full proteasomes (Kriegenburg et al., 2008; Funakoshi et al., 2009; Le Tallec et al., 2009; Park et al.,

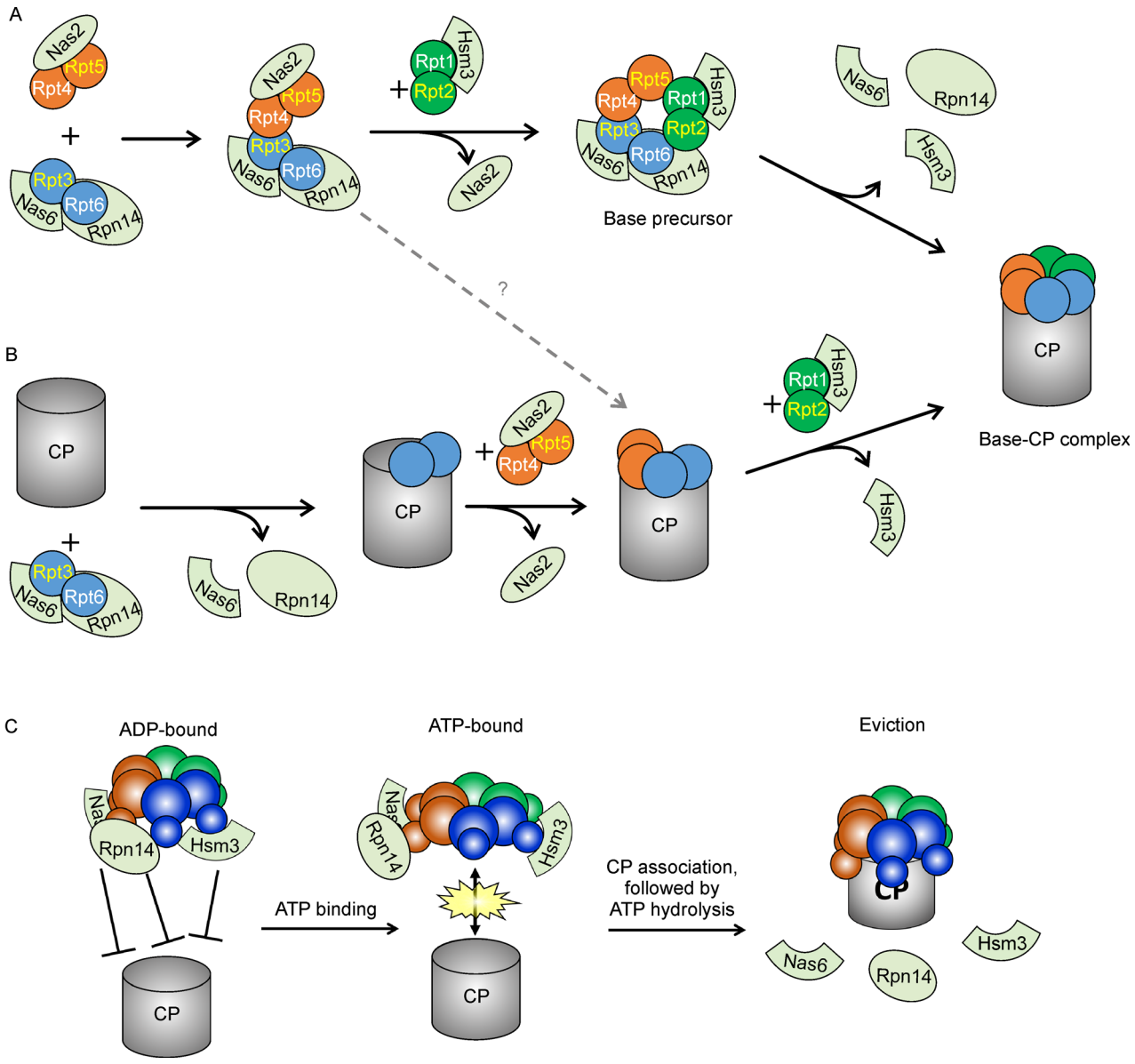

Figure 7 Overview of base assembly and chaperone eviction. Non-ATPase subunits are omitted for clarity. (A) and (B), Two non-exclusive pathways have been proposed for assembly of the RP base. In the first (A), the base forms independently of the CP. In the second (B), The CP acts as a template or scaffold for the incoming chaperone modules, and each chaperone is released as its respective module docks onto the CP. A gray dotted arrow with question mark indicates the as-yet untested possibility of crosstalk between these two proposed pathways. (C) Proposed mechanism of coupling between ATP hydrolysis and chaperone eviction by the base. The base assumes "down" or "out" conformations according to the nature of the nucleotide bound (ADP-bound vs. ATP-bound). In the ADP-bound, "down" state, the AAA + small domains (shown as small circles) that are bound by the chaperones point downward, generating steric clash (T-bars) between the chaperones and the CP. In the ATP-bound state, the chaperones are positioned outward, relieving steric hindrance and allowing formation of a metastable chaperone-base-CP complex. Subsequent ATP hydrolysis forcefully repositions the small domains to the down position, which shears the chaperones from the small domains (eviction). Although a full base is shown in this model, the same concept could in principle allow for shearing of chaperones from ATPase-active intermediates as they dock on the CP (B).

2009; Roelofs et al., 2009; Saeki et al., 2009), led to an initial model in which the three chaperone-bound base modules assemble and subsequently associate with the CP. In support of such a model, studies in mammalian cells identified complexes very similar to the Hsm3, Nas2, and Rpn14/Nas6 modules that, when mixed, would form a complex containing all subunits of the base, and that had ATPase activity (Thompson et al., 2009). Similarly, immunoprecipitation experiments in yeast demonstrated that Nas2 readily copurified all components of the Nas2 and Rpn14/Nas6 modules, but no components of the Hsm3 module, lid, or CP (Tomko et al., 2010). This implied an ordered association of modules, and suggested that exit of Nas2 was coupled to entrance of the Hsm3 module to complete base assembly

prior to CP and lid binding. An analogous stepwise incorporation was inferred in mammalian cells on the basis of intermediates that accumulated upon RNAi knockdown of base assembly chaperones (Kaneko et al., 2009). However, in this model the Nas2 module, rather than the Hsm3 module, was the last to enter the assembling base. Regardless, the stepwise nature of base assembly and the absence of the CP in all reported base assembly intermediates was otherwise consistent with findings in yeast. Later, the yeast base subcomplex, complete with assembly chaperones, was successfully produced in *E. coli* by coexpression of the nine base subunits and the four constitutive base assembly chaperones (Beckwith et al., 2013). As *E. coli* is devoid of proteasomes and associated proteins, this effort served to define the minimal chaperone requirement for base assembly and provided unequivocal evidence that the base can assemble independent of the CP (or lid).

In the CP-templated model of base assembly, base modules are delivered to the CP, and completion of the base occurs on the surface of the CP α ring. Such a role was first proposed based on the observation that base assembly intermediates accumulated when the surface of the CP α-ring was altered via deletion of the α3 subunit or Pba3-4 chaperone, which yields proteasomes containing a second α4 subunit in the place of the α3 subunit (Kusmierczyk et al., 2008) (Fig. 6). A second critical clue pointing toward a role for the CP came from studies in which the most C-terminal residue of each ATPase was systematically deleted (Park et al., 2009). Truncation of the tails of Rpt4 and Rpt6 unexpectedly resulted in strong base assembly defects, whereas the assembly of the CP and lid were unaffected. Truncation of these tails led to accumulation of the Hsm3 module, and based on these observations the authors proposed that docking of the Rpt4 and Rpt6 tails into the CP is a major driving force in base assembly *in vivo*. In support of such a role, the C-terminal tails of chaperone-bound ATPases were shown to be critical for release of the chaperones from the assembled base upon association with the CP—altering the length of the tail by only a few amino acids promoted the retention of assembly chaperones on the base, even in the presence of the CP (Park et al., 2009). The authors proposed that, in this way, the association of the base and CP serves as a regulatory mechanism to eject the chaperones once their cognate assembly intermediate has stably docked onto the CP.

Both models are in agreement that chaperones must dissociate from the RP after base assembly to properly dock to and activate the CP, as none of the chaperones have ever been identified in complex with normal, mature 26S proteasomes. The eviction of chaperones appears to be tightly coupled to association with the CP and with ATP hydrolysis by the base. In cryo-electron microscopy (cryo-EM) structures of the proteasome prepared in the presence of the non-hydrolyzable ATP analog ATPγS (which mimics an ATP-bound state), the C-terminal domains recognized by the chaperones assume an "out" position in which they are extended radially from the center of the ring (Fig. 7C, ATP-bound) (Sledz et al., 2013). In contrast, these domains are anticipated to adopt a "down" position upon ATP hydrolysis (Fig. 7C, ADP-bound) based on an analogous structure prepared in the presence of ATP, which is readily hydrolyzed, yielding a proteasome with ADP (and potentially some ATP) bound (Lasker et al., 2012; Unverdorben et al., 2014). In the down position, molecular modeling of the chaperone-bound ATPase ring indicates substantial steric clash with the CP (Roelofs et al., 2009; Park et al., 2013). However, in the ATP-bound state, the repositioning of the C-terminal domains of the ATPase subunits to the outward position would likely allow docking of the chaperone-bound base onto the CP. Subsequent ATP hydrolysis would then be anticipated to shear the chaperones from the ATPase ring upon transition to the down state (Fig. 7C, Eviction). Indeed, provision of the nonhydrolyzable ATP analog ATPγS allows the stable formation of a ternary chaperone-base-CP complex (Park et al., 2013), and studies on intermediates of the base purified from mammals indicated that base intermediates are incapable of hydrolyzing ATP (Thompson et al., 2009), and importantly, once they are reconstituted to form the base, ATPase activity is stimulated. Thus, the base appears to utilize ATP-dependent conformational changes to drive eviction of the chaperones and subsequently allow stable association with the CP.

Intrinsic mechanisms guiding proteasome assembly

Although chaperones play an integral part in the efficient and faithful assembly of the proteasome from its cognate subunits, the subunits themselves also govern their own assembly, and in several cases, transiently function to drive assembly forward. Intriguingly, the majority of these intrinsic regulatory features seem to be unique to eukaryotic proteasomes, consistent with an increased requirement for mechanisms to control biogenesis in a compositionally and architecturally more complicated structure. In many cases, these intrinsic regulatory features take the form of flexible and/or disordered appendages of subunits, which either make critical contacts with their neighbors during assembly, or serve to shield critical activities during the assembly process.

Propeptides of β subunits

The three proteolytically active β subunits (β1, β2, β5) and two non-active β subunits (β6, β7) are expressed as cleavable proproteins; a recent study provides exquisite detail on the actual mechanism of autocatalytic propeptide processing (Huber et al., 2016). These propeptides also fulfill several roles during assembly. They protect the proteolytic subunits from N-terminal acetylation on the catalytic threonines of the catalytic subunits, which would inactivate them (Arendt and

Hochstrasser, 1999; Groll et al., 1999; Jager et al., 1999). The propeptides of β5 are essential for viability, and their deletion impairs CP assembly at similar points in yeast and mammals (Chen and Hochstrasser, 1996; Hirano et al., 2008). In the former, β5 lacking its propeptide (β5Δpro) fails to incorporate into the 13S whereas in the latter, β5Δpro incorporates into the 13S but fails to recruit the next subunit, β6. The β5 propeptides are large enough (75 amino acids in yeast) to form independently functioning units; this is evidenced by suppression of lethality due to deletion of the propeptide when it is expressed *in trans* as a separate polypeptide (Chen and Hochstrasser, 1996; Jager et al., 1999). It is not clear if the β5 propeptide adopts any defined structure, although it has been suggested that part of the β5 propeptide may protrude out of the β-ring in a 15S intermediate (Kock et al., 2015; Li et al., 2016); this would be consistent with its postulated role of helping two half-proteasomes to dimerize (Li et al., 2007). Deletion of *UMP1* in yeast can also suppress the lethality of the β5 propeptide deletion (Ramos et al., 1998). This suggests that Ump1and β5 propeptide functions are linked, perhaps antagonistically. Consistent with this, human Ump1 can bind to the propeptide of β5 directly (Heink et al., 2005), though such direct interaction has not been demonstrated in yeast. One suggested possibility, at least in yeast, is that the β5 propeptide overcomes the inhibitory (i.e. checkpoint) function of Ump1 on the dimerization of two half-proteasomes (Li et al., 2007; Kock et al., 2015).

The β2 propeptide (29 amino acids in yeast) is not required for viability in yeast, although deleting it results in very strong growth defects under heat stress (Arendt and Hochstrasser, 1999; Jager et al., 1999). However, it is essential in a mammalian cell model, where it is required for β3 incorporation (Hirano et al., 2008). The yeast β2 propeptide has some ability to function *in trans* and its deletion results in processing defects of the β5 and β7 subunit propeptides (Chen and Hochstrasser, 1996; Jager et al., 1999). The β1 propeptide (19 amino acids in yeast) is dispensable for viability in yeast and mammalian cells, although like β2, it also contributes to β5 processing (Chen and Hochstrasser, 1996; Jager et al., 1999; Hirano et al., 2008). Synergistic defects when the propeptides of β1 and β2 are deleted simultaneously (Arendt and Hochstrasser, 1999) argue for concerted roles in assembly. The β7 propeptide (41 amino acids in yeast) is not essential (Jager et al., 1999) and is partially processed during assembly by β2, leaving an eight amino acid extension (i.e. a segment upstream of the Gly-1/Thr1 cleavage site which normally exposes the catalytic threonine) (Groll et al., 1999). Its role in assembly is not known. The β6 propeptide (28 amino acids in yeast) is also partially processed during assembly by β2, leaving a nine amino acid extension (Groll et al., 1999). However, this propeptide does have a role to play in assembly. A partial deletion of the propeptide, up until the nine amino acid extension, has no effect on viability but it does suppress the lethality of β5Δpro (Li et al., 2007). Complete deletion of the propeptide is lethal, but can be rescued by deletion of *UMP1* (Li et al., 2007). This is reminiscent of the effect of *UMP1* deletion on β5Δpro and suggests that the β6 propeptide, like the β5 propeptide, may help to overcome an inhibitory effect of Ump1.

Tails of β subunits

The C-termini of certain β subunits play key roles during assembly. The β2 subunit has a long tail (~30 amino acids) that wraps around β3, its neighbor in the β ring (Groll et al., 1997; Unno et al., 2002). This tail is essential in yeast and mammalian cells; its absence results in the failure to incorporate β3 (Ramos et al., 2004; Hirano et al., 2008). As it wraps around β3, the β2 tail also makes contacts with the next β subunit, β4. This likely contributes to the stability of, and ability to isolate, the 13S intermediate which contains these three β subunits bound to a complete α-ring (Li et al., 2007). The β7 subunit has a ~19 amino acid tail that inserts between the β1 and β2 subunits of the opposite β ring (Groll et al., 1997; Unno et al., 2002). Consequently, it is required for processing of the β1 propeptide and for β1 catalytic activity (Ramos et al., 2004). Moreover, it serves as a brace that helps hold two β-rings together in the CP. Its absence results in accumulation of the 15S intermediate, meaning that it is also important for β7 insertion – the rate limiting step of CP formation (Ramos et al., 2004; Li et al., 2007; Marques et al., 2007; Hirano et al., 2008). The β7 tail likely functions in concert with the β5 propeptide in helping to bring two half-proteasomes together; β7 is a high-copy suppressor of the lethality of β5Δpro, but only if its tail is present (Li et al., 2007; Li et al., 2016).

Features of α subunits

With a few differences, the tertiary folds of α subunits and β subunits are essentially superimposable (Lowe et al., 1995; Groll et al., 1997). This conservation reflects their common evolutionary origin (Volker and Lupas, 2002). The most notable difference occurs at the N terminus. Whereas β subunits contain propeptides of varying length and relatively poor sequence conservation, all α subunits contain N-terminal extensions. These extensions of ~35 amino acids include a highly conserved H0 helix which is important for α subunit assembly (Zwickl et al., 1994). The presence of the H0 helix helps explain why α subunits, but not β subunits, can assemble into rings. Striking examples of this can be found when certain eukaryotic α subunits, expressed in bacteria, assemble into single, double, and higher order rings (Gerards et al., 1997; Gerards et al., 1998;Yao et al., 1999; Ishii et al., 2015). Since not all α subunits form rings on their own, H0 are not the only determinants that contribute to α subunit assembly. The available binding energy resulting from the considerable buried surface area between eukaryotic α subunit pairs within a ring (>2500 $Å^2$) also contributes to α ring formation (Kwon et al., 2004b), as do stabilizing salt

bridges (Panfair et al., 2015). What is not known is how all of these features combined contribute to the order in which α subunits assemble to form an α-ring.

Intrinsic features regulating assembly and incorporation of the lid

Sem1 as a molecular tether during lid assembly

Similar to the CP, the lid relies on unstructured protein domains to serve as stabilizing factors during assembly. This has been best documented for the lid subunit Sem1/Rpn15 (DSS1 in humans), which serves as a molecular tether to stabilize an otherwise unstable assembly intermediate until it can be efficiently incorporated into the assembling lid (Tomko and Hochstrasser, 2014). Sem1 is an unusual proteasome subunit, with an exceptionally small size (~10 kDa) and a near-complete lack of secondary and tertiary protein structure (Kragelund et al., 2016). Aside from a C-terminal α-helix, Sem1 contains no well-defined protein fold, and consists of two highly conserved binding domains separated by an unstructured linker sequence (Fig. 8A). The two binding domains are rich in acidic residues, which are important for recognition of their binding partners. The first binding site, constituting residues 29-45 in yeast, recognizes a positively charged crevice in the proteasomal Rpn3 lid subunit (Fig. 8B) (Wei et al., 2008; Dambacher et al., 2016). Sem1 is able to bind Rpn3 in the absence of any other proteins, and may serve as its folding or stabilizing chaperone (Tomko and Hochstrasser, 2014). In addition to conserved acidic residues, the second site in Sem1 contains two conserved tryptophan residues that dock Sem1 into hydrophobic pockets in the lid. These tryptophan residues make critical contacts with Rpn3 and Rpn7 (Fig. 8B, inset), and are necessary for stable binding to Rpn3 or Rpn7 (Tomko and Hochstrasser, 2014; Dambacher et al., 2016).

Although Rpn3 and Rpn7 interact extensively in the fully assembled proteasome and in the isolated lid, these proteins display poor affinity for one another in isolation, implying that their stable association relies on a remodeling of their interaction surfaces during assembly, or that they are stabilized by interactions between additional subunits in the context of larger assembly products (Tomko and Hochstrasser, 2014). During lid biogenesis, Sem1 binds these two subunits and stabilizes their otherwise weak interaction to yield the trimeric lid assembly intermediate LP3 (Fig. 3A). This tethering role was supported by experiments demonstrating that separation of the two binding sites, via expression of Sem1 as two fragments split through the linker region, failed to promote LP3 formation. Similarly, a mutant Sem1 harboring an extended linker sequence readily promoted LP3 formation, whereas a mutant form with a shortened linker region did not, indicating a minimal reach between Sites 1 and 2 is required for its function. The flexible linker region in Sem1 between sites 1 and 2 is both disordered and poorly conserved, which allowed engineering of a protease cleavage site into it without disrupting the assembly function of Sem1. Using this cleavable form of Sem1, the tethering function of Sem1 was assessed in a variety of proteasomal assembly intermediates (Tomko and Hochstrasser, 2014). Importantly, cleavage of Sem1 in the context of LP3 resulted in dissociation of Rpn3 and Rpn7, consistent with the proposed tethering role, but once these subunits had incorporated into larger complexes, Sem1 could be cleaved with no apparent loss of interaction between Rpn3 and Rpn7. Together, this indicated that the tethering role of Sem1 was important only during the initial stages of lid assembly, and that this role becomes dispensable once Rpn3 and Rpn7 are incorporated into higher order intermediates, consistent with a model where their interface is stabilized via remodeling and/or interactions with other subunits within the lid.

Sem1 is somewhat promiscuous among proteasome subunits—it has been shown to be an integral component of other multisubunit protein complexes, including the TREX-2 mRNA export complex, and a complex containing the BRCA-2 tumor suppressor (reviewed in (Kragelund et al., 2016)). The binding sites on Sem1 that associate with the proteasome overlap substantially with those used to associate with these complexes, which suggests first that Sem1 can likely associate with only one of these structures at a time, but also, that Sem1 may be reversibly recruited from one complex to another to control assembly or function of these respective complexes. Whether Sem1 serves as an assembly chaperone for these other complexes has not been studied in great detail, but it is known that DSS1 functions to stabilize BRCA-2 akin to Sem1 with Rpn3 (Li et al., 2006). Sem1 has recently been reported to bind ubiquitin using binding site 2 (Paraskevopoulos et al., 2014), although this role has been disputed (Shi et al., 2016). Nonetheless, under some circumstances, occupation of this site by ubiquitin may also serve to fine-tune the assembly or function of the proteasome indirectly via Sem1.

Lid subunit C-terminal helices

The use of avidity via multiple binding interactions is also utilized more broadly within the proteasomal lid to drive assembly. Each lid subunit, save for Sem1, contributes C-terminal α-helices to an unusual helical bundle (Estrin et al., 2013). In the context of lid assembly, this helical bundle also serves to drive the stepwise assembly of the lid from its cognate subunits by generating avid binding surfaces. These surfaces recruit subsequent subunits to the nascent complex. An elegant study using heterologously expressed lid subunits in *E. coli* demonstrated that this helical bundle is a critical determinant of the lid subunit assembly sequence (Estrin et al., 2013). By systematically coexpressing a truncated lid subunit lacking its C-terminal helix with the other eight subunits and assessing lid assembly by gel filtration, a tentative assembly sequence congruent with the available

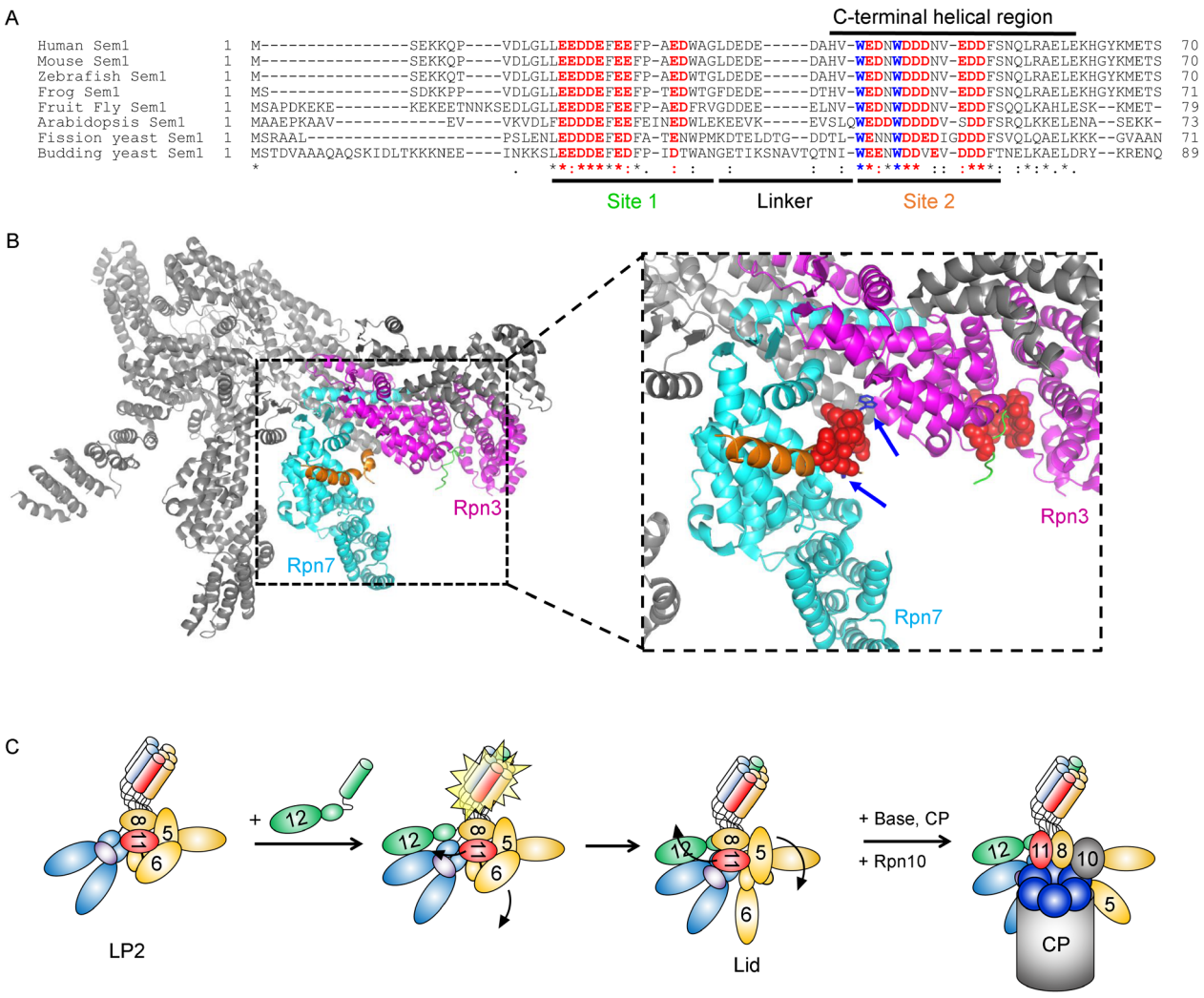

Figure 8 Intrinsic regulatory features of the lid important for proteasome biogenesis. (A) Sequence alignment of select Sem1/DSS1 homologs. Conserved binding regions Site 1 and Site 2, as well as the poorly conserved linker region and indicated below the alignment. The region that forms a helix in available EM structures of the proteasome is indicated above the alignment. The acidic residues characteristic of Site 1 and Site 2 are highlighted in red, whereas the conserved tryptophan residues present in Site 2 are highlighted in blue. (B) The cryo-EM structure of the isolated lid from yeast (PDB ID 3JCK) is shown, highlighting the positioning of Sem1 between Rpn3 and Rpn7. Lid subunits Rpn5, 6, 8, 9, 11, and 12 are colored gray, whereas Rpn3 and Rpn7 are colored magenta and cyan, respectively. Only portions of Sem1 are clearly resolved in this structure, but they are shown as green and orange. Site 1 is located within the green segment, and Site 2 within the orange. Inset, conserved acidic residues in Site 1 and Site 2 are shown in space-filling mode as spheres to illustrate their roles in docking Sem1 onto Rpn3 and Rpn7. Conserved tryptophan residues are shown in stick mode in blue and indicated with blue arrows. (C) Conformational changes associated with lid assembly and attachment to the base. In a low-resolution EM structure of LP2, the N-termini of Rpn6 (and potentially Rpn5) are closed inward toward the Rpn8-Rpn11 heterodimer. Lid subunit coloring is the same as in Fig. 3A.

literature was inferred, and demonstrated a clear requirement for the lid subunit C-terminal helices for efficient assembly.

An integrative modeling approach revealed that these C-terminal helices form a helical bundle, similar to the cylinder of a revolver. In this bundle the central helix, contributed by Rpn12, is surrounded by the others. The Rpn12 helix makes numerous weak contacts with helices contributed by the majority of subunits within both Module 1 and LP3. Thus, the C-terminal helix of Rpn12 likely "senses" the assembly state of the lid by virtue of its dependence on numerous weak interactions with residues contributed by the helices of most other lid subunits (Tomko and Hochstrasser, 2011).

As the lid assembles independently of dedicated chaperones, it must similarly rely on intrinsic features to regulate joining to the base. Despite the fact that the lid buries an enormous amount of surface area in the base and CP, most studies indicate that intermediates of the lid, including an intermediate called LP2 that lacks only the Rpn12 subunit, have no appreciable affinity for the base or CP *in vitro* (Tomko and Hochstrasser, 2011; Tomko et al., 2015). In

agreement, blockade of lid assembly via genetic means in yeast leads to the accumulation of lid intermediates, all of which are devoid of base or CP (Fukunaga et al., 2010; Tomko and Hochstrasser 2011). A major unanswered question thus has been: what mechanism restrains lid attachment until completion of lid assembly? Recent investigations into the structures of the isolated lid and lid assembly intermediates have implicated several conformational changes as pivotal maturation events that permit completion of RP assembly.

Recently, a combination of quantitative crosslinking-mass spectrometry (QCL-MS) and negative stain EM reported that the LP2 intermediate undergoes substantial conformational rearrangement upon incorporation of Rpn12 that in turn permits its efficient assembly into the proteasome holoenzyme (Tomko et al., 2015). In contrast to the structure of the proteasome-associated lid, LP2 adopts a more compact state in which the N-termini of several subunits appear to move inward toward the PCI horseshoe and the Rpn8-11 heterodimer, similar to a closed fist (Fig. 8C). Importantly, provision of the conserved C-terminal helix of Rpn12 alone was sufficient to drive this conformational reorganization and RP formation (Fig. 8C), implicating engagement of the lid helical bundle as the critical determinant of this large scale conformational shift.

A second layer of control lies in a conformational change in the position of the Rpn8-Rpn11 deubiquitinating module within the lid (Dambacher et al., 2016). The cryo-EM structure of the isolated lid unexpectedly revealed that the Rpn8-Rpn11 module is positioned approximately perpendicular to the orientation observed in the full proteasome. In this position, it is collapsed inward toward the core of the lid, and is cradled by contacts with the neighboring lid subunits Rpn5 and Rpn9. This conformation is highly reminiscent of that observed in the low-resolution EM structure of LP2 and thus is likely sterically incompatible with the base (Tomko et al., 2015). Thus, at least two critical conformational changes are necessary for lid-base association—a repositioning of Rpn5 and Rpn6 N-termini that are folded inward toward the core of the lid, occluding the base, and rotation of the Rpn8-Rpn11 module into an extended conformation. Further mechanistic studies will be essential to clarify how these important structural transitions occur during RP biogenesis.

Maturation of RP enzymatic activities

Within the proteasome holoenzyme, substrate binding, unfolding, deubiquitination, and proteolysis are tightly coupled. Decoupling of these activities would result in the counterproductive return of deubiquitinated or unfolded protein substrates to the cellular milieu without degradation, which could disrupt cellular processes or initiate formation of toxic protein inclusions. Because enzymatic coupling of proteasomal activities is dependent on the proper engagement and communication between proteasomal subcomplexes, it is imperative that the activities of isolated subcomplexes or intermediates be suppressed during biogenesis, and that they mature successfully upon complete assembly of the holoenzyme. The processing of the β subunit propeptides (described above) is one example whereby a catalytic activity of the proteasome is restrained until it is safely contained within the proteolytic chamber at the interior of the CP.

In the past two years, much progress has been made in understanding maturation of the lid's Rpn11-dependent deubiquitinating activity. At least two mechanisms restrict Rpn11 activity until incorporation of the lid into the proteasome. The first is an autoinhibitory mechanism present in the closed conformation observed in the isolated lid and, potentially, intermediates (Tomko et al., 2015; Dambacher et al., 2016). In this conformation where the Rpn8-Rpn11 is collapsed inward, the Rpn11 active site is shielded from the environment. Importantly, the side chain of Rpn5-Asn274 intrudes into the Rpn11 active site where it displaces a water molecule necessary for catalysis. The repositioning of the Rpn8-Rpn11 module upon incorporation of the lid into the proteasome exposes the Rpn11 active site and frees it from inhibition by Rpn5, permitting catalysis.

A second mechanism restricting Rpn11 activity prior to completion of proteasome assembly relies upon physical separation of Rpn11 from its substrates, and exploits the unique nature of Rpn11 among deubiquitinating enzymes to accomplish this. Whereas most DUBs either trim polyubiquitin chains from the distal end or cleave between Ub moieties, Rpn11 cleaves the isopeptide bond between the most proximal ubiquitin and the substrate itself, releasing polyubiquitin chains *en bloc* (Verma et al., 2002; Yao and Cohen, 2002). Rpn11 must therefore tolerate substantial variability in the substrate sequence surrounding the scissile Ub isopeptide bond, and does so by making little to no contact with the substrate (Pathare et al., 2014; Worden et al., 2014). Although this yields very poor substrate affinity in isolation, this is compensated for in the proteasome via high affinity binding of the substrate's polyubiquitin chain by proteasomal ubiquitin receptors contained within the base, and the pulling of the isopeptide bond into the active site of Rpn11 by the base ATPases during substrate unfolding and translocation. Thus, Rpn11 activity is restrained during assembly by virtue of its physical separation from its major substrate recruitment mechanisms. We posit that this may be particularly important early during the assembly of the lid, in the context of intermediates that do not yet contain Rpn5 to bind Rpn11 and exclude the catalytic water as observed in the isolated lid structure.

As mentioned above, the ability of the proteasomal ATPases to hydrolyze ATP appears to be inhibited in the context of assembly intermediates, and is activated upon completion of base assembly. The mechanism by which ATPase activity is restrained until completion of base assembly is unknown, but likely depends upon critical interactions of the ATPase subunits with neighboring

ATPases to orient nucleotide properly for catalysis (Kim et al., 2015), which would serve to couple completion of the ATPase ring to maturation of ATPase activity. The fully assembled base contains ubiquitin receptors and unfolding activity, even in the absence of the lid and CP. Thus, it can in principle recruit and unfold substrates in isolation, decoupling unfolding from deubiquitination or proteolysis. Unfolding of substrates without deubiquitination or degradation is repressed by the activity of the Ubp6 deubiquitinase (Sakata et al., 2011), which is found associated with the earliest Rpt1- and Rpt2-containing base assembly intermediates and is present on the base and the full proteasome. Ubp6 abuts the ATPase ring (Aufderheide et al., 2015; Bashore et al., 2015), and can deubiquitinate substrates that are recruited to base assembly intermediates (Sakata et al., 2011). Although deubiquitination of proteasome substrates without degradation may be unproductive, it may be less likely to promote toxicity than spurious unfolding and release of unfolded proteins that could then aggregate and cause toxicity. The level of free base subcomplexes in the cell is very low based on estimates from yeast cell extracts (Funakoshi et al., 2009); it could be that the cell can tolerate a small amount of spurious substrate deubiquitination at the expense of sparing protein aggregation and the resultant toxicity. Alternatively, the base has been reported to have refolding activity, at least in the context of the 26S proteasome or base-CP complexes (Braun et al., 1999). It is possible that this chaperone-like function is active in the context of assembly intermediates to guard against spurious unfolding and subsequent release of any substrates that engage the assembling RP.

Emerging themes in proteasome assembly

It remains important to continue expanding our knowledge of intrinsic features of proteasome components, and the extrinsic factors that act upon them. However, it is also clear that proteasome assembly is more than the sum of these parts. Below we present some of the frontiers that will give rise to the next chapters in our understanding of the biogenesis of this essential molecular machine.

Quality control of failed assembly products

The degradation of individual polypeptides by the UPS is well understood, whereas the mechanisms mediating degradation of the proteasome itself or proteasomal subcomplexes are only recently coming to light (Marshall et al., 2015; Peters et al., 2015; Marshall et al., 2016). Studies of proteasome assembly in various organisms have revealed that formation of non-native or off-pathway assembly products can and does occur. But, the nature and fate of those assembly products has not yet been addressed in great detail. A clearance pathway is almost certainly necessary for such species, and perhaps compounding the difficulty of this task, these species may not display obvious features that demarcate them as “damaged” in the same way a misfolded protein does, necessitating distinct mechanisms to recognize and clear them.

Recent investigations of proteasome quality control mechanisms have revealed a novel pathway of Atg8-mediated proteasome autophagy, termed proteaphagy (Marshall et al., 2015), which has since been demonstrated in several species (Cohen-Kaplan et al., 2016; Waite et al., 2016). Proteaphagy was first observed in *Arabidopsis* as accumulation of proteasome subunits in autophagy (atg) mutants with compromised ubiquitin-like protein Atg8 lipidation or autophagic induction by Atg1 kinase. This was shown to be due to autophagic turnover of proteasomes. Proteaphagy could be stimulated by nitrogen starvation or by treatment of cells with the noncovalent proteasome inhibitor MG132. The pathways mediating proteasome turnover in response to these two stimuli appeared to be distinct—the Atg1 kinase responsible for nutrient sensing was essential only in proteaphagy induced through nitrogen starvation, whereas Atg8 was required for proteaphagy induced by both nitrogen starvation and MG132. The association of ubiquitin receptor RPN10 was found to increase concomitantly with proteasome inhibition, accompanied by an increase in proteasome-associated ubiquitination. RPN10, which binds both Atg8 and ubiquitin, was identified as the tether between ubiquitinated proteasomes and Atg8 to facilitate the targeting of proteasomes to autophagic membranes and subsequent vacuolar degradation. However, Atg8-RPN10 interaction requires a ubiquitin-interacting motif within RPN10 that is absent in some species. The ubiquitin receptor protein Cue5 was identified as the functional equivalent of RPN10 in yeast, binding both Atg8 and the ubiquitinated proteasome complex (Marshall et al., 2016). Together, this data identified the first pathway capable of destroying fully assembled proteasomes.

Although the observation that treatment with proteasome inhibitors stimulates proteasome turnover is clear, it remains unclear whether this pathway specifically serves a true proteasome quality control function. The inhibitor utilized, MG132, is a noncovalent and reversible inhibitor, indicating that irreversible inactivation is not necessary to stimulate proteaphagy. Also, the induction of proteaphagy is greatly delayed compared to the rapid proteasome inhibition by MG132 (Marshall et al., 2016). Rather, we suggest that this turnover may instead represent “collateral damage” to proteasomes resulting from enhanced nonselective macroautophagy in response to proteasome inhibition. Indeed, numerous studies have observed upregulation of the basal autophagy rate to compensate for proteasome inhibition (Iwata et al., 2005; Ding et al., 2007; Pandey et al., 2007; Hoang et al., 2009; Zhu et al., 2010). Nonetheless, this pathway remains a highly attractive mechanism to dispose of irreversibly damaged or misassembled complexes containing proteasome subunits.

Recently, misfolded proteasome subunits have been reported to accumulate in insoluble protein deposits (IPODs), which are thought to serve as intermediate compartments for these subunits. In some cases, normal subunits were also recruited, raising the possibility that partially assembled, defective complexes were delivered to these IPODs. Small heat shock protein Hsp42, an important factor in IPOD assembly, appears to function as an essential chaperone for IPOD delivery, as *hsp42*Δ precludes proteasome subunit localization in IPODs and abolishes autophagic clearance of proteasome subunits (Marshall et al., 2015; Peters et al., 2015). Proteasomes do not colocalize with IPODs under normal conditions, therefore suggesting the relationship between IPODs and proteasomes is a quality control mechanism (Kaganovich et al., 2008). It has been suggested that Hsp42-mediated sequestration of proteasome subunits to IPODs is an alternative to a more favored pathway involving the UPS-mediated degradation of its own misassembled subunits.

An additional mechanism that may control proteasome quality is via binding and inactivation of structurally defective proteasomes by Ecm29. Ecm29 is an evolutionarily conserved protein consisting primarily of HEAT repeats that associates only with proteasomes containing both RP and CP complexes (Leggett et al., 2002). Ecm29 appears to be selectively recruited to proteasomes that harbor a defect at the interface between the CP and the RP (Lee et al., 2011; Park et al., 2011; De La Mota-Peynado et al., 2013). In yeast, mutations to the HbYX motif of Rpt5, or to the pocket lysine residues in α subunits, has been shown to enhance the association of Ecm29. Importantly, lysate-mixing experiments clearly demonstrated that Ecm29 preferentially associated with structurally defective proteasomes over normal proteasomes (Lehmann et al., 2010).

Ecm29 appears to contact both the CP and the RP, based on EM, crosslinking, and biochemical studies (Leggett et al., 2002; Lehmann et al., 2010; De La Mota-Peynado et al., 2013; Wani et al., 2016), and recent enzymatic experiments demonstrated clearly that it functions in part to suppress the catalytic activity of the ATPase ring (De La Mota-Peynado et al., 2013). In this manner, Ecm29 may serve to suppress the activity of functionally defective proteasomes that may otherwise interfere with degradation of proteasome substrates. Recently, it was shown that phosphorylation of the α7 subunit of the CP serves as a major recruiting signal for Ecm29 (Wani et al., 2016). This represents the first example of phosphorylation-dependent recruitment of a proteasome-interacting protein to the proteasome, and points toward a potential signaling mechanism for marking defective proteasomes. Two important questions persist regarding this finding: first, the kinase responsible for this phosphorylation event has not yet been determined. Second, whether such phosphorylation is constitutively present on proteasomes is unknown. Understanding the latter question would serve to clarify whether the structural defect serves to recruit the kinase for phosphorylation, or if instead the phosphorylation sites are only accessible to Ecm29 in proteasomes harboring a structural defect.

The role of subunit expression stoichiometry in assembly efficiency and fidelity

As alluded to earlier, subunit heterogeneity in eukaryotes brings with it additional complications to efficient macromolecular assembly. Two of these are order of assembly and, given the structural similarities between subunits, the potential for subunit mispairing. These two can influence each other and are thus not mutually exclusive. We've discussed above how assembly factors and intrinsic features of subunits can mitigate these difficulties. A third level of complexity is the stoichiometry of the individual components. The levels at which proteasome subunits are expressed can impact the assembly pathway followed, as well as the composition and abundance of the assembly products. Control of proteasome levels is best understood in yeast.

In yeast, proteasome levels are regulated at the transcriptional level by Rpn4. This C2H2 zinc finger motif protein recognizes conserved PACE (proteasome-associated control element) sequences in the promoters of proteasome genes (Mannhaupt et al., 1999; Xie and Varshavsky, 2001), though some deviation from the canonical 9 base pair PACE consensus is observed (Shirozu et al., 2015). Rpn4 is turned over rapidly by the proteasome via ubiquitin-dependent and-independent mechanisms (Xie and Varshavsky 2001; Ju and Xie, 2004). When proteasome activity is compromised, Rpn4 levels rise leading to increased production of proteasome subunits. This is followed by increased assembly of proteasomes which, in turn, resume the rapid Rpn4 turnover, leading to a downregulation of further subunit synthesis. In mammals, there is no Rpn4 homolog but proteasome subunit levels are also coordinately regulated (Meiners et al., 2003), in an analogous regulatory loop, by the transcription factor Nrf1 (Radhakrishnan et al., 2010). This protein is an ER-targeted glycoprotein (Wang and Chan, 2006; Zhang et al., 2007) that is constitutively turned over by the proteasome in a Cdc48-dependent fashion (Radhakrishnan et al., 2014). When proteasome function is compromised, Nrf1 levels accumulate and the protein is proteolytically cleaved by the proteasome itself (Sha and Goldberg, 2014), or by an aspartyl protease (Koizumi et al., 2016; Lehrbach and Ruvkun, 2016), prior to activating proteasome gene expression.

Though not the only means by which proteasome subunit levels are regulated, these elegant feedback mechanisms help maintain proteasome subunits at approximately stoichiometric levels, which in yeast have been variously estimated to be between 10000 to 30000 copies per cell for different subunits (Russell et al., 1999; Ghaemmaghami et al., 2003; Kulak et al., 2014). However, there are important future considerations hiding behind this apparent stoichiometry. First, these numbers refer to total subunit amounts, the vast

majority of which exist in fully assembled proteasomes. For understanding assembly, it is the level of unincorporated subunits that will be relevant, but much more difficult to determine. Second, suppose we assume that the two-to-threefold variation in total subunit levels is reflected in the variation (i.e. two-to-threefold) of unincorporated subunit levels, these small differences may be more than enough to influence the order of subunit assembly (and misassembly). An interesting analysis of complex haploinsufficiency interactions (CHI) in yeast found associations between heterozygous deletion of the yeast actin gene *ACT1* and heterozygous deletions of essential proteasome genes (Haarer et al., 2011). The obvious implication is that the proteasome regulates actin dynamics. But beyond this simple interpretation is the intriguing observation that not all proteasome genes exhibited CHI with actin. In the CP, only α6, α7, α1, β2, β4, and β5 exhibited CHI with actin. In the RP base, only Rpt2 did so, whereas all of the RP lid subunits (except Sem1) exhibited CHI with actin. The authors hypothesized that this was due to assembly defects caused when the levels of some of the subunits became limiting (Haarer et al., 2011). This example highlights the need for experiments aimed at investigating the relationship between subunit levels, stoichiometry, and assembly efficiency/fidelity.

Localization in assembly

Proteasomes are present in the nucleus and cytoplasm of mammalian cells in roughly comparable proportions (Reits et al., 1997). In yeast, proteasomes are concentrated in the nucleus (and nuclear periphery) (Enenkel et al., 1998; Russell et al., 1999). The concentration of 26S proteasomes is estimated to be just under 1 μM in the yeast nucleus and about 5-fold less in the cytoplasm (Pack et al., 2014). Remarkably similar values for the mean cytoplasmic concentration (~190 nM) of 26S proteasomes were found in mammalian neurons (Asano et al., 2015). The question of where assembly takes place has generated many suggestions, but no single agreed upon model. Certain CP and RP subunits have nuclear localization sequences (NLS) (Tanaka et al., 1990; Nederlof et al., 1995; Wendler et al., 2004); hence any assembly intermediates that contain them should, in theory, be transportable. Consistent with this, isolated base, lid, and CP have all been reported to be capable of import into the nucleus independently of each other (Wang et al., 1997; Mayr et al., 1999; Wendler et al., 2004; Isono et al., 2007). Some have reported import of CP occurring with Rpn1, Rpn2, Hsp90, and importin β in a *Xenopus* extract assay (Savulescu et al., 2011), while others implicate a role for Blm10 in helping import CP (Weberruss et al., 2013). It has even been argued that CP precursors are imported into the yeast nucleus, where assembly is completed (Lehmann et al., 2002), and alternatively that full 26S proteasomes are imported into the yeast nucleus, thereby arguing that assembly can proceed to completion in the cytoplasm (Pack et al., 2014). The distribution of the various CP and RP assembly factors throughout the cell does not help in resolving the question of assembly location, though Ump1 appears to be primarily nuclear (Lehmann et al., 2002; Huh et al., 2003; Hoefer et al., 2006; Le Tallec et al., 2007; Saeki et al., 2009).

An interesting observation placed human Ump1 directly in contact with membranes and serving to recruit CP assembly intermediates to the ER (Fricke et al., 2007). Moreover, CP assembly defects were observed when TRC40 or Bag6, two proteins involved in the pathway which inserts tail-anchored proteins into the mammalian ER membrane, were knocked down (Akahane et al., 2013). Specifically, β subunits were poorly incorporated and this correlated with poor recruitment of CP assembly intermediates to the ER membrane. Strong genetic interactions between proteasome assembly factors and yeast components of the tail-anchoring pathway were also observed, suggesting a conserved mechanism involving CP assembly at ER membranes might be at play (Akahane et al., 2013). The same study also reported that Bag6 was required for the stability of the Nas2 module as Bag6 knockdowns resulted in accumulation of RP-like species lacking Rpt4 and Rpt5 (Akahane et al., 2013), suggesting Bag6 might have multiple roles in proteasome assembly. However, the question of where individual steps of assembly might occur awaits a firm conclusion.

Assembly as a regulatory mechanism – making proteasomes for the job at hand

The ability to modify proteasomes for different functions is not a new concept. One simple way to envision modification is through subunit substitution. The discovery and characterization of mammalian immunoproteasomes, containing inducible paralogs (β1i, β2i, β5i) in place of the constitutive catalytically-active subunits (β1, β2, β5), was an important part of the foundation upon which the field of proteasome biogenesis was built ((Driscoll et al., 1993; Gaczynska et al., 1993) and reviewed in (Basler et al., 2013)). For example, the identification of the first proteasome assembly intermediates (Frentzel et al., 1994; Nandi et al., 1997; Schmidtke et al., 1997) and some key studies on the roles of β subunit propeptides were carried out on immunoproteasomes (Griffin et al., 1998; Kingsbury et al., 2000; De et al., 2003). Immunoproteasomes also provide an excellent example of how assembly can be used as a regulatory mechanism to generate alternate versions of a multi-protein complex suited for different tasks. For a recent review of immunoproteasome structure and function, we direct the readers here (Basler et al., 2013). Below, we will briefly discuss how assembly proceeds when multiple paralogs are present in the same cell. In the case of the immune β subunits, there is a different order of β subunit assembly due to cooperative recruitment among the inducible subunits (Griffin et al., 1998). The β1i subunit enters the α-ring early, versus β1 which is a "late" β subunit, and recruits β2i (Groettrup et al., 1997; Hirano et al., 2008).

This is followed by β3, β4, and β5i (Bai et al., 2014). The key is that the presence of β1i and β2i facilitates the recruitment of β5i (Kingsbury et al., 2000), which can occur even after β3, in the absence of β4 (Bai et al., 2014). Moreover, the propeptides of β2i and β5i specifically direct entry of these subunits into immunoproteasomes; this specificity is transplantable, as demonstrated by elegant peptide swap experiments (Kingsbury et al., 2000; De et al., 2003). Thus intrinsic features of paralogous subunits can influence assembly. This is further confirmed by the third mammalian β5 paralog, the thymus specific β5t that gives rise to thymoproteasomes (Murata et al., 2007), whose propeptide endows it with an ability to outcompete the constitutive β5 subunit as well (Bai et al., 2014). However, extrinsic factors are also important, as human Ump1 preferentially binds to β5i, over β5, and directs its assembly into immunoproteasomes (Heink et al., 2005).

In addition to paralogy, there are other means of using assembly as a regulatory mechanism for functional specialization. One was introduced earlier in the form of alternative α4–α4 proteasomes which are found conserved between yeast and humans (Velichutina et al., 2004; Kusmierczyk et al., 2008; Padmanabhan et al., 2016). It is still not understood how these proteasomes are assembled, or what regulates the process. In human cells, phosphorylation of the α4 subunit prevents its ubiquitin-dependent degradation leading to increased levels of this subunit (Li et al., 2015); as α4 levels rise, increased levels of α4–α4 proteasomes form (Padmanabhan et al., 2016). Thus mass action, in the form of an increased α4/α3 ratio (and/or an increased α4/PAC3-PAC4 ratio), may be sufficient to allow formation of α4–α4 proteasomes (Padmanabhan et al., 2016). Notably, in yeast the only CP assembly factor that lacks either the canonical PACE elements for the Rpn4 transcription factor, or the minimum Rpn4-responsive elements (PACE-core), is Pba3-Pba4 (Shirozu et al., 2015). Thus, conditions that stabilize Rpn4, and lead to upregulation of proteasome subunits, should not significantly impact Pba3-Pba4 levels. This also sets up a potential mass-action scenario whereby an increased α4/Pba3-Pba4 ratio could lead to increased α4–α4 proteasome formation. Experiments are underway to test this hypothesis.

The functional significance of α4–α4 proteasomes is not yet known. However, generation of α4–α4 proteasomes creates a CP lacking the major gating subunit and thus should be constitutively open (Groll et al., 2000); it also gives rise to a different α-ring that could impact the binding of RP or other activators (see below). Either, or both, of these features could contribute to the role of α4–α4 proteasomes in the cell. Whether replacement of α3 with α4 is the only example of non-paralogous subunit substitution within the proteasome remains to be seen, though this is easier to envision occurring with structurally homologous subunits (such as subunits of the CP) or when the substituted subunit is not essential, like α3.

Another means of using assembly to regulate proteasome function is via the use of alternative regulators that bind the CP. This review is focused on the canonical 26S proteasome, in which a CP is bound by one or more RP complexes. However, it bears mention that CP can interact with a host of other protein complexes, most with demonstrated ability to stimulate the catalytic activity of the CP. These include the aforementioned Blm10/PA200, which is broadly conserved across the eukarya (Ustrell et al., 2002; Schmidt et al., 2005), and members of the 11S family of activators (also called PA28, or REG) found primarily in higher eukaryotes. The latter includes the heteroheptameric PA28αβ (or REGαβ) complex and the homoheptameric PA28γ (or REGγ). The functions of the 11S complexes are not well understood (for recent reviews, readers are directed here (Mao et al., 2008; Stadtmueller and Hill, 2011; Cascio, 2014)) but they can cap CP independently, or as hybrid complexes with RP. Though they lack HbYX motifs, the C-termini of 11S complexes insert into the same inter α-subunit pockets used by other activators (Whitby et al., 2000; Forster et al., 2005). In addition, PI31, originally identified as an inhibitor of CP in vitro (Chu-Ping et al., 1992), represents a fourth class of CP regulator conserved from yeast to mammals (Zaiss et al., 2002; Bader et al., 2011; Hatanaka et al., 2011; Li et al., 2014; Yashiroda et al., 2015). Phylogenomic analysis suggests that all four classes of regulator (RP, Blm10/PA200, 11S, and PI31) were present in the last eukaryotic common ancestor, despite the subsequent loss of some these proteins in many descendant lineages (Fort et al., 2015). Finally, there is evidence that the archaeal ortholog of Cdc48 forms degradation-competent complexes with archaeal CP, though if the same is true of eukaryotic Cdc48 remains to be conclusively demonstrated (Barthelme and Sauer, 2012a; Barthelme and Sauer, 2013; Barthelme et al., 2014; Barthelme et al., 2015).

Regardless of which combination of regulators is present in a given eukaryotic cell, the fact that they all likely share a conserved binding mode with CP on the outer α-ring surface means that they must compete for this surface. The existence of two such α-ring surfaces on each CP also makes hybrid complexes possible, as mentioned above. Consequently, questions about the function of these CP-regulator complexes (especially the various possible hybrids) are also ultimately questions of assembly and the regulation thereof. To illustrate this point, recent work by Welk and colleagues (2016) demonstrated differential recruitment of various regulators, in response to proteasome inhibition, thereby generating alternatively-capped proteasomes (Welk et al., 2016).

Disruptions to proteasome assembly in human disease

Although numerous studies have tied changes in proteasome activity to human disease, the exact molecular mechanisms underpinning breakdowns in proteasomal proteolysis remain poorly studied. Recent advances in DNA sequencing have yielded a wealth of information about genetic variations in the genomes of diseased tissues. These approaches have

uncovered a substantial number of genetic changes and single nucleotide polymorphisms within proteasome subunit or assembly chaperone genes (reviewed in (Gomes, 2013)). In the majority of cases, the potential effects of these polymorphisms are unknown. However, several recent studies have provided evidence that disruption of proteasome assembly results from polymorphisms in proteasome subunits or assembly chaperones, and may represent an underlying cause of several human diseases. We highlight three particularly compelling examples here.

Nakajo-Nishimura syndrome is a rare autosomal recessive inflammatory disorder characterized by periodic fever, skin rashes, joint contractures, and lipomuscular atrophy. A recent sequencing effort unveiled a single nucleotide transversion (c.602G>T) within the coding sequence of the *PSMB8* gene (Arima et al., 2011). Strikingly, this variant was present in all patients tested, immediately implicating it in disease pathology. *PSMB8* encodes the β5i subunit of the immunoproteasome, which has important roles in the innate and adaptive immune systems, and this mutation results in a coding sequence mutation of Gly201 to valine. This glycine residue is highly conserved, and its mutation to valine disrupts immunoproteasome assembly. The resultant loss of proteasome activity due to reduced assembly of immunoproteasomes was associated with increased inflammatory cytokine production in peripheral lymphocytes, providing strong evidence that this variant is responsible, or at least a major contributing factor, to the disease pathology. Intriguingly, a second variant in *PSMB8* observed in a related inflammatory disease, JMP syndrome (Agarwal et al., 2010), reduces chymotryptic activity without disturbing proteasome assembly. This suggests that disruption of immunoproteasome function via mutations to *PSMB8* may define a related class of autoinflammatory diseases, and indicates that disruption of immunoproteasome assembly can be as pathogenic as mutations directly impacting proteolysis.

A second example derives from studies of an Italian family with a documented history of type II diabetes (T2D). Sequencing a chromosomal region identified via classical genetic mapping as being linked to T2D led to the identification of a haplotype containing two intronic variants and a coding variant of the *PSMD9* gene (Gragnoli and Cronsell, 2007), which encodes the human ortholog of the Nas2 base assembly chaperone. This coding variant results in a missense mutation of Glu197 to glycine. Modeling of this residue position onto the available crystal structures of Nas2 from yeast (Singh et al., 2014) indicates this residue lies in the terminal strand of a β-sheet that makes up the core of the PDZ domain. Glycine is known to favor disorder, further suggesting that this mutation may disrupt the structure, and in turn the function, of human Nas2, causing predisposition of carriers to T2D. In support of a role for alterations in proteasome assembly in T2D etiology, a second disease-associated variant in the immediate upstream sequence of *PSMA6*, which encodes the α1 subunit of the CP, is associated with T2D in the Chinese Dongxiang and Han populations (Liu et al., 2012). This variant, −8C>G, is within the Kozak consensus sequence of the *PSMA6* gene, strongly implying it results in an alteration in subunit translation. The effects of α1 subunit depletion, as well as the effect of this mutation on CP assembly *in vivo*, have not been empirically tested, but this circumstantial evidence again supports a link between defects in proteasome assembly and human disease that awaits evaluation.

A study of European patients with Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome (KLICK) syndrome provides a compelling third example. KLICK syndrome is a rare autosomal recessive disease, characterized by ichthyosis, palmoplantar keratoderma with constricting bands around fingers, flexural deformities of fingers, and keratotic papules in a linear distribution on the flexural side of large joints (Dahlqvist et al., 2010). Genome-wide SNP analysis revealed a single-nucleotide deletion at position c. – 95 in the proteasome maturation protein (POMP) gene, the human ortholog of Ump1, present in all patients tested. This deletion was accompanied by a redistribution of POMP, as well as proteasomal subunits α7 and β5. The redistribution occurs during the formation of the horny layer of the epidermis, whose thickening is a hallmark pathology of KLICK syndrome, suggesting that the disease is caused by proteasome insufficiency at a specific stage of epidermal differentiation.

Together, these pioneering examples provide strong evidence that disruption of proteasome assembly could yield pathological outcomes as severe as direct interference with proteasome function. Considering the large number of genomic variations in proteasome subunits and assembly chaperones that have been recently reported (Gomes 2013; Lek et al., 2016), it will be important in the future to test how these variations affect proteasome assembly and resultant cellular proteolytic capacity. The recent publication of atomic resolution structures of the human proteasome (Huang et al., 2016; Schweitzer et al., 2016) will serve as powerful tools to guide *in silico* and *in vivo* attempts to identify variants most likely to impact proteasome biogenesis *in vivo*.

Perspectives

Now that a basic understanding of the factors and possible proteasome assembly pathways has emerged, it has become clear that proteasome biogenesis depends critically on a combination of extrinsic factors and intrinsic features for efficient formation *in vivo*. Emerging paradigms include roles for the dedicated assembly chaperones in restricting the possible arrangements of subunits within ring-based structures, as well as roles for intrinsically disordered regions, in the forms of tails, propeptides, or in some cases, entire subunits, to reinforce metastable, transient intermediates. With this newfound understanding, many new questions

arise. Major uncertainties that are coming to the forefront include the relative contributions of redundant assembly pathways *in vivo*, as well as how their use may change under conditions of increased proteasome biogenesis, in which some assembly chaperones may become limiting while others accumulate. Similarly, it will be important to understand how assembly may be compromised in human diseases characterized by breakdown in protein quality control, such as Alzheimer's disease, Parkinson's disease, or type II diabetes. We posit that the advent of next-generation sequencing of large collections of disease-associated tissues will continue to reveal mutations predicted to disrupt the proteasome assembly network, and could point toward new targets for intervention in diseases impacted by changes to proteasome biogenesis or function.

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Compliance with ethics guidelines

Lauren A. Howell, Robert J. Tomko Jr. and Andrew R. Kusmierczyk declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Agarwal A K, Xing C, DeMartino G N, Mizrachi D, Hernandez M D, Sousa A B, Martínez de Villarreal L, dos Santos H G, Garg A (2010). PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. Am J Hum Genet, 87(6): 866–872

Akahane T, Sahara K, Yashiroda H, Tanaka K, Murata S (2013). Involvement of Bag6 and the TRC pathway in proteasome assembly. Nat Commun, 4: 2234

Arendt C S, Hochstrasser M (1997). Identification of the yeast 20S proteasome catalytic centers and subunit interactions required for active-site formation. Proc Natl Acad Sci USA, 94(14): 7156–7161

Arendt C S, Hochstrasser M (1999). Eukaryotic 20S proteasome catalytic subunit propeptides prevent active site inactivation by N-terminal acetylation and promote particle assembly. EMBO J, 18(13): 3575–3585

Arima K, Kinoshita A, Mishima H, Kanazawa N, Kaneko T, Mizushima T, Ichinose K, Nakamura H, Tsujino A, Kawakami A, Matsunaka M, Kasagi S, Kawano S, Kumagai S, Ohmura K, Mimori T, Hirano M, Ueno S, Tanaka K, Tanaka M, Toyoshima I, Sugino H, Yamakawa A, Tanaka K, Niikawa N, Furukawa F, Murata S, Eguchi K, Ida H, Yoshiura K (2011). Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. Proc Natl Acad Sci USA, 108 (36): 14914–14919

Asano S, Fukuda Y, Beck F, Aufderheide A, Forster F, Danev R, Baumeister W (2015). Proteasomes. A molecular census of 26S proteasomes in intact neurons. Science, 347(6220): 439–442

Aufderheide A, Beck F, Stengel F, Hartwig M, Schweitzer A, Pfeifer G, Goldberg A L, Sakata E, Baumeister W, Förster F (2015). Structural characterization of the interaction of Ubp6 with the 26S proteasome. Proc Natl Acad Sci USA, 112(28): 8626–8631

Bader M, Benjamin S, Wapinski O L, Smith D M, Goldberg A L, Steller H (2011). A conserved f box regulatory complex controls proteasome activity in *Drosophila*. Cell, 145(3): 371–382

Bai M, Zhao X, Sahara K, Ohte Y, Hirano Y, Kaneko T, Yashiroda H, Murata S (2014). Assembly mechanisms of specialized core particles of the proteasome. Biomolecules, 4(3): 662–677

Barrault M B, Richet N, Godard C, Murciano B, Le Tallec B, Rousseau E, Legrand P, Charbonnier J B, Le Du M H, Guerois R, Ochsenbein F, Peyroche A (2012). Dual functions of the Hsm3 protein in chaperoning and scaffolding regulatory particle subunits during the proteasome assembly. Proc Natl Acad Sci USA, 109(17): E1001–E1010

Barthelme D, Chen J Z, Grabenstatter J, Baker T A, Sauer R T (2014). Architecture and assembly of the archaeal Cdc48•20S proteasome. Proc Natl Acad Sci USA, 111(17): E1687–E1694

Barthelme D, Jauregui R, Sauer RT (2015). An ALS disease mutation in Cdc48/p97 impairs 20S proteasome binding and proteolytic communication. Protein Sci, 24:1521–1527

Barthelme D, Sauer R T (2012a). Identification of the Cdc48•20S proteasome as an ancient AAA + proteolytic machine. Science, 337 (6096): 843–846

Barthelme D, Sauer RT (2012b). Identification of the Cdc48•20S proteasome as an ancient AAA + proteolytic machine. Science, 337 (6096): 843–846

Barthelme D, Sauer R T (2013). Bipartite determinants mediate an evolutionarily conserved interaction between Cdc48 and the 20S peptidase. Proc Natl Acad Sci USA, 110(9): 3327–3332

Bashore C, Dambacher C M, Goodall E A, Matyskiela M E, Lander G C, Martin A (2015). Ubp6 deubiquitinase controls conformational dynamics and substrate degradation of the 26S proteasome. Nat Struct Mol Biol, 22(9): 712–719

Basler M, Kirk C J, Groettrup M (2013). The immunoproteasome in antigen processing and other immunological functions. Curr Opin Immunol, 25(1): 74–80

Beck F, Unverdorben P, Bohn S, Schweitzer A, Pfeifer G, Sakata E, Nickell S, Plitzko J M, Villa E, Baumeister W, Forster F (2012). Near-atomic resolution structural model of the yeast 26S proteasome. Proc Natl Acad Sci USA, 109(37): 14870–14875

Beckwith R, Estrin E, Worden E J, Martin A (2013). Reconstitution of the 26S proteasome reveals functional asymmetries in its AAA + unfoldase. Nat Struct Mol Biol, 20(10): 1164–1172

Benaroudj N, Goldberg A L (2000). PAN, the proteasome-activating nucleotidase from archaebacteria, is a protein-unfolding molecular chaperone. Nat Cell Biol, 2(11): 833–839

Braun B C, Glickman M, Kraft R, Dahlmann B, Kloetzel P M, Finley D, Schmidt M (1999). The base of the proteasome regulatory particle exhibits chaperone-like activity. Nat Cell Biol, 1(4): 221–226

Burri L, Hockendorff J, Boehm U, Klamp T, Dohmen R J, Levy F

(2000). Identification and characterization of a mammalian protein interacting with 20S proteasome precursors. Proc Natl Acad Sci USA, 97(19): 10348–10353

Cascio P (2014). PA28alphabeta: the enigmatic magic ring of the proteasome? Biomolecules, 4(2): 566–584

Chen P, Hochstrasser M (1996). Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly. Cell, 86(6): 961–972

Chu-Ping M, Slaughter C A, DeMartino G N (1992). Purification and characterization of a protein inhibitor of the 20S proteasome (macropain). Biochim Biophys Acta, 1119(3): 303–311

Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, Kwon Y T, Ciechanover A (2016). p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. Proc Natl Acad Sci USA, 113(47): E7490–E7499

Colot H V, Park G, Turner G E, Ringelberg C, Crew C M, Litvinkova L, Weiss R L, Borkovich K A, Dunlap J C (2006). A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. Proc Natl Acad Sci USA, 103(27): 10352–10357

da Fonseca P C, He J, Morris E P (2012). Molecular Model of the Human 26S Proteasome. Mol Cell, 46(1): 54–66

Dahlqvist J, Klar J, Tiwari N, Schuster J, Törmä H, Badhai J, Pujol R, van Steensel M A M, Brinkhuizen T, Gijezen L, Chaves A, Tadini G, Vahlquist A, Dahl N (2010). A single-nucleotide deletion in the POMP 5′ UTR causes a transcriptional switch and altered epidermal proteasome distribution in KLICK genodermatosis. Am J Hum Genet, 86(4): 596–603

Dambacher C M, Worden E J, Herzik M A, Martin A, Lander G C (2016). Atomic structure of the 26S proteasome lid reveals the mechanism of deubiquitinase inhibition. eLife, 5: e13027

Dange T, Smith D, Noy T, Rommel P C, Jurzitza L, Cordero R J B, Legendre A, Finley D, Goldberg A L, Schmidt M (2011). Blm10 protein promotes proteasomal substrate turnover by an active gating mechanism. J Biol Chem, 286(50): 42830–42839

De M, Jayarapu K, Elenich L, Monaco J J, Colbert R A, Griffin T A (2003). Beta 2 subunit propeptides influence cooperative proteasome assembly. J Biol Chem, 278(8): 6153–6159

De La Mota-Peynado A, Lee S Y, Pierce B M, Wani P, Singh C R, Roelofs J (2013). The proteasome-associated protein Ecm29 inhibits proteasomal ATPase activity and *in vivo* protein degradation by the proteasome. J Biol Chem, 288(41): 29467–29481

DeMartino G N, Proske R J, Moomaw C R, Strong A A, Song X, Hisamatsu H, Tanaka K, Slaughter C A (1996). Identification, purification, and characterization of a PA700-dependent activator of the proteasome. J Biol Chem, 271(6): 3112–3118

Ding W X, Ni H M, Gao W, Yoshimori T, Stolz D B, Ron D, Yin X M (2007). Linking of autophagy to ubiquitin-proteasome system is important for the regulation of endoplasmic reticulum stress and cell viability. Am J Pathol, 171(2): 513–524

Driscoll J, Brown M G, Finley D, Monaco J J (1993). MHC-linked LMP gene products specifically alter peptidase activities of the proteasome. Nature, 365(6443): 262–264

Enenkel C, Lehmann A, Kloetzel P M (1998). Subcellular distribution of proteasomes implicates a major location of protein degradation in the nuclear envelope-ER network in yeast. EMBO J, 17(21): 6144–6154

Estrin E, Lopez-Blanco J R, Chacon P, Martin A (2013). Formation of an Intricate Helical Bundle Dictates the Assembly of the 26S Proteasome Lid. Structure, 21(9): 1624–1635

Fehlker M, Wendler P, Lehmann A, Enenkel C (2003). Blm3 is part of nascent proteasomes and is involved in a late stage of nuclear proteasome assembly. EMBO Rep, 4(10): 959–963

Finley D (2009). Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem, 78(1): 477–513

Forouzan D, Ammelburg M, Hobel C F, Stroh L J, Sessler N, Martin J, Lupas A N (2012). The archaeal proteasome is regulated by a network of AAA ATPases. J Biol Chem, 287(46): 39254–39262

Forster A, Masters E I, Whitby F G, Robinson H, Hill C P (2005). The 1.9 A structure of a proteasome-11S activator complex and implications for proteasome-PAN/PA700 interactions. Mol Cell, 18 (5): 589–599

Fort P, Kajava A V, Delsuc F, Coux O (2015). Evolution of proteasome regulators in eukaryotes. Genome Biol Evol, 7(5): 1363–1379

Frentzel S, Pesold-Hurt B, Seelig A, Kloetzel P M (1994). 20 S proteasomes are assembled via distinct precursor complexes. Processing of LMP2 and LMP7 proproteins takes place in 13–16 S preproteasome complexes. J Mol Biol, 236(4): 975–981

Fricke B, Heink S, Steffen J, Kloetzel P M, Kruger E (2007). The proteasome maturation protein POMP facilitates major steps of 20S proteasome formation at the endoplasmic reticulum. EMBO Rep, 8 (12): 1170–1175

Fukunaga K, Kudo T, Toh-e A, Tanaka K, Saeki Y (2010). Dissection of the assembly pathway of the proteasome lid in Saccharomyces cerevisiae. Biochem Biophys Res Commun, 396(4): 1048–1053

Funakoshi M, Tomko R J Jr, Kobayashi H, Hochstrasser M (2009). Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base. Cell, 137(5): 887–899

Gaczynska M, Rock K L, Goldberg A L (1993). Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. Nature, 365(6443): 264–267

Gerards W L, Enzlin J, Häner M, Hendriks I LA M, Aebi U , Bloemendal H, Boelens W (1997). The human alpha-type proteasomal subunit HsC8 forms a double ringlike structure, but does not assemble into proteasome-like particles with the beta-type subunits HsDelta or HsBPROS26. J Biol Chem, 272(15): 10080–10086

Gerards W L, de Jong W W, Bloemendal H, Boelens W (1998). The human proteasomal subunit HsC8 induces ring formation of other alpha-type subunits. J Mol Biol, 275(1): 113–121

Ghaemmaghami S, Huh W K, Bower K, Howson R W, Belle A, Dephoure N, O'Shea E K, Weissman J S (2003). Global analysis of protein expression in yeast. Nature, 425(6959): 737–741

Gille C, Goede A, Schlöetelburg C, Preißner R, Kloetzel P M, Göbel U B, Frömmel C (2003). A comprehensive view on proteasomal sequences: implications for the evolution of the proteasome. J Mol Biol, 326(5): 1437–1448

Gillette T G, Kumar B, Thompson D, Slaughter C A, DeMartino G N (2008). Differential roles of the COOH termini of AAA subunits of PA700 (19 S regulator) in asymmetric assembly and activation of the 26 S proteasome. J Biol Chem, 283(46): 31813–31822

Gomes A V (2013). Genetics of proteasome diseases. Scientifica (Cairo), 2013: 637629

Gragnoli C, Cronsell J (2007). PSMD9 gene variants within NIDDM2 may rarely contribute to type 2 diabetes. J Cell Physiol, 212(3): 568–571

Griffin T A, Nandi D, Cruz M, Fehling H J, Kaer L V, Monaco J J, Colbert R A (1998). Immunoproteasome assembly: cooperative incorporation of interferon gamma (IFN-gamma)-inducible subunits. J Exp Med, 187(1): 97–104

Griffin T A, Slack J P, McCluskey T S, Monaco J J, Colbert R A (2000). Identification of proteassemblin, a mammalian homologue of the yeast protein, Ump1p, that is required for normal proteasome assembly. Mol Cell Biol Res Commun, 3(4): 212–217

Groettrup M, Standera S, Stohwasser R, Kloetzel P M (1997). The subunits MECL-1 and LMP2 are mutually required for incorporation into the 20S proteasome. Proc Natl Acad Sci USA, 94(17): 8970–8975

Groll M, Brandstetter H, Bartunik H, Bourenkow G, Huber R (2003). Investigations on the maturation and regulation of archaebacterial proteasomes. J Mol Biol, 327(1): 75–83

Groll M, Ditzel L, Löwe J, Stock D, Bochtler M, Bartunik H D, Huber R (1997). Structure of 20S proteasome from yeast at 2.4 A resolution. Nature, 386(6624): 463–471

Groll M, Glickman M H, Finley D, Bajorek M, Köhler A, Moroder L, Rubin D M, Huber R (2000). A gated channel into the proteasome core particle. Nat Struct Biol, 7(11): 1062–1067

Groll M, Heinemeyer W, Jager S, Ullrich T, Bochtler M, Wolf D H, Huber R (1999). The catalytic sites of 20S proteasomes and their role in subunit maturation: a mutational and crystallographic study. Proc Natl Acad Sci USA, 96(20): 10976–10983

Haarer B, Aggeli D, Viggiano S, Burke D J, Amberg D C (2011). Novel interactions between actin and the proteasome revealed by complex haploinsufficiency. PLoS Genet, 7(9): e1002288

Hanssum A, Zhong Z, Rousseau A, Krzyzosiak A, Sigurdardottir A, Bertolotti A (2014). An inducible chaperone adapts proteasome assembly to stress. Mol Cell, 55(4): 566–577

Hatanaka A, Chen B, Sun J Q, Mano Y, Funakoshi M, Kobayashi H, Ju Y, Mizutani T, Shinmyozu K, Nakayama J, Miyamoto K, Uchida H, Oki M (2011). Fub1p, a novel protein isolated by boundary screening, binds the proteasome complex. Genes Genet Syst, 86(5): 305–314

Heinemeyer W, Fischer M, Krimmer T, Stachon U, Wolf D H (1997). The active sites of the eukaryotic 20 S proteasome and their involvement in subunit precursor processing. J Biol Chem, 272(40): 25200–25209

Heink S, Ludwig D, Kloetzel P M, Kruger E (2005). IFN-gamma-induced immune adaptation of the proteasome system is an accelerated and transient response. Proc Natl Acad Sci USA, 102 (26): 9241–9246

Hirano Y, Hayashi H, Iemura S, Hendil K B, Niwa S, Kishimoto T, Kasahara M, Natsume T, Tanaka K, Murata S (2006). Cooperation of multiple chaperones required for the assembly of mammalian 20S proteasomes. Mol Cell, 24(6): 977–984

Hirano Y, Hendil K B, Yashiroda H, Iemura S, Nagane R, Hioki Y, Natsume T, Tanaka K, Murata S (2005). A heterodimeric complex that promotes the assembly of mammalian 20S proteasomes. Nature, 437(7063): 1381–1385

Hirano Y, Kaneko T, Okamoto K, Bai M, Yashiroda H, Furuyama K, Kato K, Tanaka K, Murata S (2008). Dissecting beta-ring assembly pathway of the mammalian 20S proteasome. EMBO J, 27(16): 2204–2213

Hoang B, Benavides A, Shi Y, Frost P, Lichtenstein A (2009). Effect of autophagy on multiple myeloma cell viability. Mol Cancer Ther, 8 (7): 1974–1984

Hoefer M M, Boneberg E M, Grotegut S, Kusch J, Illges H (2006). Possible tetramerisation of the proteasome maturation factor POMP/proteassemblin/hUmp1 and its subcellular localisation. Int J Biol Macromol, 38(3-5): 259–267

Huang X, Luan B, Wu J, Shi Y (2016). An atomic structure of the human 26S proteasome. Nat Struct Mol Biol, 23(9): 778–785

Huber E M, Heinemeyer W, Li X, Arendt C S, Hochstrasser M, Groll M (2016). A unified mechanism for proteolysis and autocatalytic activation in the 20S proteasome. Nat Commun, 7: 10900

Huh W K, Falvo J V, Gerke L C, Carroll A S, Howson R W, Weissman J S, O'Shea E K (2003). Global analysis of protein localization in budding yeast. Nature, 425(6959): 686–691

Ishii K, Noda M, Yagi H, Thammaporn R, Seetaha S, Satoh T, Kato K, Uchiyama S (2015). Disassembly of the self-assembled, double-ring structure of proteasome alpha7 homo-tetradecamer by alpha6. Sci Rep, 5: 18167

Isono E, Nishihara K, Saeki Y, Yashiroda H, Kamata N, Ge L, Ueda T, Kikuchi Y, Tanaka K, Nakano A, Toh-e A (2007). The assembly pathway of the 19S regulatory particle of the yeast 26S proteasome. Mol Biol Cell, 18(2): 569–580

Iwata A, Riley B E, Johnston J A, Kopito R R (2005). HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin. J Biol Chem, 280(48): 40282–40292

Jager S, Groll M, Huber R, Wolf D H, Heinemeyer W (1999). Proteasome beta-type subunits: unequal roles of propeptides in core particle maturation and a hierarchy of active site function. J Mol Biol, 291(4): 997–1013

Ju D, Xie Y (2004). Proteasomal degradation of RPN4 via two distinct mechanisms, ubiquitin-dependent and-independent. J Biol Chem, 279(23): 23851–23854

Kaganovich D, Kopito R, Frydman J (2008). Misfolded proteins partition between two distinct quality control compartments. Nature, 454(7208): 1088–1095

Kaneko T, Hamazaki J, Iemura S, Sasaki K, Furuyama K, Natsume T, Tanaka K, Murata S (2009). Assembly pathway of the Mammalian proteasome base subcomplex is mediated by multiple specific chaperones. Cell, 137(5): 914–925

Kim D U, Hayles J, Kim D, Wood V, Park H O, Won M, Yoo H S, Duhig T, Nam M, Palmer G, Han S, Jeffery L, Baek S T, Lee H, Shim Y S, Lee M, Kim L, Heo K S, Noh E J, Lee A R, Jang Y J, Chung K S, Choi S J, Park J Y, Park Y, Kim H M, Park S K, Park H J, Kang E J, Kim H B, Kang H S, Park H M, Kim K, Song K, Song K B, Nurse P, Hoe K L (2010a). Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. Nat Biotechnol, 28(6): 617–623

Kim S, Saeki Y, Fukunaga K, Suzuki A, Takagi K, Yamane T, Tanaka K, Mizushima T, Kato K (2010b). Crystal structure of yeast rpn14, a chaperone of the 19 S regulatory particle of the proteasome. J Biol Chem, 285(20): 15159–15166

Kim Y C, Snoberger A, Schupp J, Smith D M (2015). ATP binding to neighbouring subunits and intersubunit allosteric coupling underlie proteasomal ATPase function. Nat Commun, 6(8520):1

Kingsbury D J, Griffin T A, Colbert R A (2000). Novel propeptide function in 20 S proteasome assembly influences beta subunit composition. J Biol Chem, 275(31): 24156–24162

Kleijnen M F, Roelofs J, Park S, Hathaway N A, Glickman M, King R W, Finley D (2007). Stability of the proteasome can be regulated allosterically through engagement of its proteolytic active sites. Nat Struct Mol Biol, 14(12): 1180–1188

Kloetzel P M (2004). Generation of major histocompatibility complex class I antigens: functional interplay between proteasomes and TPPII. Nat Immunol, 5(7): 661–669

Kock M, Nunes M M, Hemann M, Kube S, Jürgen Dohmen R, Herzog F, Ramos P C, Wendler P (2015). Proteasome assembly from 15S precursors involves major conformational changes and recycling of the Pba1-Pba2 chaperone. Nat Commun, 6: 6123

Koizumi S, Irie T, Hirayama S, Sakurai Y, Yashiroda H, Naguro I, Ichijo H, Hamazaki J, Murata S (2016). The aspartyl protease DDI2 activates Nrf1 to compensate for proteasome dysfunction. eLife, 5: e18357

Kragelund B B, Schenstrom S M, Rebula C A, Panse V G, Hartmann-Petersen R (2016). DSS1/Sem1, a multifunctional and intrinsically disordered protein. Trends Biochem Sci, 41(5): 446–459

Kriegenburg F, Seeger M, Saeki Y, Tanaka K, Lauridsen A M B, Hartmann-Petersen R, Hendil K B (2008). Mammalian 26S proteasomes remain intact during protein degradation. Cell, 135(2): 355–365

Kulak N A, Pichler G, Paron I, Nagaraj N, Mann M (2014). Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. Nat Methods, 11(3): 319–324

Kusmierczyk A R, Hochstrasser M (2008). Some assembly required: dedicated chaperones in eukaryotic proteasome biogenesis. Biol Chem, 389(9): 1143–1151

Kusmierczyk A R, Kunjappu M J, Funakoshi M, Hochstrasser M (2008). A multimeric assembly factor controls the formation of alternative 20S proteasomes. Nat Struct Mol Biol, 15(3): 237–244

Kusmierczyk A R, Kunjappu M J, Kim R Y, Hochstrasser M (2011). A conserved 20S proteasome assembly factor requires a C-terminal HbYX motif for proteasomal precursor binding. Nat Struct Mol Biol, 18(5): 622–629

Kwon Y D, Nagy I, Adams P D, Baumeister W, Jap B K (2004a). Crystal structures of the Rhodococcus proteasome with and without its pro-peptides: implications for the role of the pro-peptide in proteasome assembly. J Mol Biol, 335(1): 233–245

Kwon Y D, Nagy I, Adams P D, Baumeister W, Jap B K (2004b). Crystal structures of the Rhodococcus proteasome with and without its pro-peptides: implications for the role of the pro-peptide in proteasome assembly. J Mol Biol, 335(1): 233–245

Lander G C, Estrin E, Matyskiela M E, Bashore C, Nogales E, Martin A (2012). Complete subunit architecture of the proteasome regulatory particle. Nature, 482: 186–191

Lasker K, Forster F, Bohn S, Walzthoeni T, Villa E, Unverdorben P, Beck F, Aebersold R, Sali A, Baumeister W (2012). Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. Proc Natl Acad Sci USA, 109(5): 1380–1387

Le Tallec B, Barrault M B, Courbeyrette R, Guerois R, Marsolier-Kergoat M C, Peyroche A (2007). 20S proteasome assembly is orchestrated by two distinct pairs of chaperones in yeast and in mammals. Mol Cell, 27(4): 660–674

Le Tallec B, Barrault M B, Guerois R, Carre T, Peyroche A (2009). Hsm3/S5b participates in the assembly pathway of the 19S regulatory particle of the proteasome. Mol Cell, 33(3): 389–399

Lee S C, Shaw B D (2007). A novel interaction between N-myristoylation and the 26S proteasome during cell morphogenesis. Mol Microbiol, 63(4): 1039–1053

Lee S Y, De la Mota-Peynado A, Roelofs J (2011). Loss of Rpt5 protein interactions with the core particle and Nas2 protein causes the formation of faulty proteasomes that are inhibited by Ecm29 protein. J Biol Chem, 286(42): 36641–36651

Leggett D S, Hanna J, Borodovsky A, Crosas B, Schmidt M, Baker R T, Walz T, Ploegh H, Finley D (2002). Multiple associated proteins regulate proteasome structure and function. Mol Cell, 10(3): 495–507

Lehmann A, Janek K, Braun B, Kloetzel P M, Enenkel C (2002). 20 S proteasomes are imported as precursor complexes into the nucleus of yeast. J Mol Biol, 317(3): 401–413

Lehmann A, Niewienda A, Jechow K, Janek K, Enenkel C (2010). Ecm29 fulfils quality control functions in proteasome assembly. Mol Cell, 38(6): 879–888

Lehrbach N J, Ruvkun G (2016). Proteasome dysfunction triggers activation of SKN-1A/Nrf1 by the aspartic protease DDI-1. eLife, 5: e17721

Lek M, Karczewski K J, Minikel E V, Samocha K E, Banks E, Fennell T, O'Donnell-Luria A H, Ware J S, Hill A J, Cummings B B, Tukiainen T, Birnbaum D P, Kosmicki J A, Duncan L E, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper D N, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki M I, Moonshine A L, Natarajan P, Orozco L, Peloso G M, Poplin R, Rivas M A, Ruano-Rubio V, Rose S A, Ruderfer D M, Shakir K, Stenson P D, Stevens C, Thomas B P, Tiao G, Tusie-Luna M T, Weisburd B, Won H H, Yu D, Altshuler D M, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez J C, Gabriel S B, Getz G, Glatt S J, Hultman C M, Kathiresan S, Laakso M, McCarroll S, McCarthy M I, McGovern D, McPherson R, Neale B M, Palotie A, Purcell S M, Saleheen D, Scharf J M, Sklar P, Sullivan P F, Tuomilehto J, Tsuang M T, Watkins H C, Wilson J G, Daly M J, MacArthur D G (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616): 285–291

Li D, Dong Q, Tao Q, Gu J, Cui Y, Jiang X, Yuan J, Li W, Xu R, Jin Y, Li P, Weaver D T, Ma Q, Liu X, Cao C (2015). c-Abl regulates proteasome abundance by controlling the ubiquitin-proteasomal degradation of PSMA7 subunit. Cell Reports, 10(4): 484–496

Li J, Zou C, Bai Y, Wazer D E, Band V, Gao Q (2006). DSS1 is required for the stability of BRCA2. Oncogene, 25(8): 1186–1194

Li X, Kusmierczyk A R, Wong P, Emili A, Hochstrasser M (2007). beta-Subunit appendages promote 20S proteasome assembly by overcoming an Ump1-dependent checkpoint. EMBO J, 26(9): 2339–2349

Li X, Li Y, Arendt C S, Hochstrasser M (2016). Distinct elements in the proteasomal beta5 subunit propeptide required for autocatalytic processing and proteasome assembly. J Biol Chem, 291(4): 1991–2003

Li X, Thompson D, Kumar B, DeMartino G N (2014). Molecular and cellular roles of PI31 (PSMF1) protein in regulation of proteasome function. J Biol Chem, 289(25): 17392–17405

Liu J, Yuan X, Liu J, Tian L, Quan J, Liu J, Chen X, Wang Y, Shi Z, Zhang J (2012). Validation of the association between PSMA6 −8 C/G polymorphism and type 2 diabetes mellitus in Chinese Dongxiang and Han populations. Diabetes Res Clin Pract, 98(2): 295–301

Lowe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R (1995). Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 A resolution. Science, 268(5210): 533–539

Luan B, Huang X, Wu J, Mei Z, Wang Y, Xue X, Yan C, Wang J, Finley D J, Shi Y, Wang F (2016). Structure of an endogenous yeast 26S proteasome reveals two major conformational states. Proc Natl Acad Sci USA, 113(10): 2642–2647

Mannhaupt G, Schnall R, Karpov V, Vetter I, Feldmann H (1999). Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. FEBS Lett, 450(1-2): 27–34

Mao I, Liu J, Li X, Luo H (2008). REGgamma, a proteasome activator and beyond? Cellular and molecular life sciences. Cell Mol Life Sci, 65: 3971–3980

Marques A J, Glanemann C, Ramos P C, Dohmen R J (2007). The C-terminal extension of the beta7 subunit and activator complexes stabilize nascent 20 S proteasomes and promote their maturation. J Biol Chem, 282(48): 34869–34876

Marshall R S, Li F, Gemperline D C, Book A J, Vierstra R D (2015). Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/ubiquitin receptor RPN10 in *Arabidopsis*. Mol Cell, 58 (6): 1053–1066

Marshall R S, McLoughlin F, Vierstra R D (2016). Autophagic turnover of inactive 26S Proteasomes in yeast is directed by the ubiquitin receptor Cue5 and the Hsp42 chaperone. Cell Reports, 16(6): 1717–1732

Matyskiela M E, Lander G C, Martin A (2013). Conformational switching of the 26S proteasome enables substrate degradation. Nat Struct Mol Biol, 20(7): 781–788

Mayr J, Seemuller E, Muller S A, Engel A, Baumeister W (1998a). Late events in the assembly of 20S proteasomes. J Struct Biol, 124(2-3): 179–188

Mayr J, Seemuller E, Muller S A, Engel A, Baumeister W (1998b). Late events in the assembly of 20S proteasomes. J Struct Biol, 124(2-3): 179–188

Mayr J, Wang H R, Nederlof P, Baumeister W (1999). The import pathway of human and Thermoplasma 20S proteasomes into HeLa cell nuclei is different from that of classical NLS-bearing proteins. Biol Chem, 380(10): 1183–1192

Meiners S, Heyken D, Weller A, Ludwig A, Stangl K, Kloetzel P M, Kruger E (2003). Inhibition of proteasome activity induces concerted expression of proteasome genes and de novo formation of Mammalian proteasomes. J Biol Chem, 278(24): 21517–21525

Murata S, Sasaki K, Kishimoto T, Niwa S, Hayashi H, Takahama Y, Tanaka K (2007). Regulation of $CD8^+$ T cell development by thymus-specific proteasomes. Science, 316(5829): 1349–1353

Nakamura Y, Umehara T, Tanaka A, Horikoshi M, Padmanabhan B, Yokoyama S (2007). Structural basis for the recognition between the regulatory particles Nas6 and Rpt3 of the yeast 26S proteasome. Biochem Biophys Res Commun, 359(3): 503–509

Nandi D, Woodward E, Ginsburg D B, Monaco J J (1997). Intermediates in the formation of mouse 20S proteasomes: implications for the assembly of precursor beta subunits. EMBO J, 16(17): 5363–5375

Nederlof P M, Wang H R, Baumeister W (1995). Nuclear localization signals of human and Thermoplasma proteasomal alpha subunits are functional *in vitro*. Proc Natl Acad Sci USA, 92(26): 12060–12064

Pack C G, Yukii H, Toh-e A, Kudo T, Tsuchiya H, Kaiho A, Sakata E, Murata S, Yokosawa H, Sako Y, Baumeister W, Tanaka K, Saeki Y (2014). Quantitative live-cell imaging reveals spatio-temporal dynamics and cytoplasmic assembly of the 26S proteasome. Nat Commun, 5: 3396

Padmanabhan A, Vuong S A, Hochstrasser M (2016). Assembly of an evolutionarily conserved alternative proteasome isoform in human cells. Cell Reports, 14(12): 2962–2974

Pandey U B, Nie Z, Batlevi Y, McCray B A, Ritson G P, Nedelsky N B, Schwartz S L, DiProspero N A, Knight M A, Schuldiner O, Padmanabhan R, Hild M, Berry D L, Garza D, Hubbert C C, Yao T P, Baehrecke E H, Taylor J P (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature, 447(7146): 859–863

Panfair D, Ramamurthy A, Kusmierczyk A R (2015). Alpha-ring independent assembly of the 20S proteasome. Sci Rep, 5: 13130

Paraskevopoulos K, Kriegenburg F, Tatham M H, Rösner H I, Medina B, Larsen I B, Brandstrup R, Hardwick K G, Hay R T, Kragelund B B, Hartmann-Petersen R, Gordon C (2014). Dss1 is a 26S proteasome ubiquitin receptor. Mol Cell, 56(3): 453–461

Park S, Kim W, Tian G, Gygi S P, Finley D (2011). Structural defects in the regulatory particle-core particle interface of the proteasome induce a novel proteasome stress response. J Biol Chem, 286(42): 36652–36666

Park S, Li X, Kim H M, Singh C R, Tian G, Hoyt M A, Lovell S, Battaile K P, Zolkiewski M, Coffino P, Roelofs J, Cheng Y, Finley D (2013). Reconfiguration of the proteasome during chaperone-mediated assembly. Nature, 497(7450): 512–516

Park S, Roelofs J, Kim W, Robert J, Schmidt M, Gygi S P, Finley D (2009). Hexameric assembly of the proteasomal ATPases is templated through their C termini. Nature, 459(7248): 866–870

Pathare G R, Nagy I, Sledz P, Anderson D J, Zhou H J, Pardon E, Steyaert J, Forster F, Bracher A, Baumeister W (2014). Crystal structure of the proteasomal deubiquitylation module Rpn8-Rpn11. Proc Natl Acad Sci USA, 111(8): 2984–2989

Peters L Z, Karmon O, David-Kadoch G, Hazan R, Yu T, Glickman M H, Ben-Aroya S (2015). The protein quality control machinery regulates its misassembled proteasome subunits. PLoS Genet, 11(4): e1005178

Radhakrishnan S K, den Besten W, Deshaies R J (2014). p97-dependent retrotranslocation and proteolytic processing govern formation of active Nrf1 upon proteasome inhibition. eLife, 3: e01856

Radhakrishnan S K, Lee C S, Young P, Beskow A, Chan J Y, Deshaies R J (2010). Transcription factor Nrf1 mediates the proteasome recovery pathway after proteasome inhibition in mammalian cells. Mol Cell, 38(1): 17–28

Ramos P C, Hockendorff J, Johnson E S, Varshavsky A, Dohmen R J (1998). Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. Cell, 92(4): 489–499

Ramos P C, Marques A J, London M K, Dohmen R J (2004). Role of C-terminal extensions of subunits beta2 and beta7 in assembly and activity of eukaryotic proteasomes. J Biol Chem, 279(14): 14323–14330

Reits E A, Benham A M, Plougastel B, Neefjes J, Trowsdale J (1997). Dynamics of proteasome distribution in living cells. EMBO J, 16 (20): 6087–6094

Roelofs J, Park S, Haas W, Tian G, McAllister F E, Huo Y, Lee B H,

Zhang F, Shi Y, Gygi S P, Finley D (2009). Chaperone-mediated pathway of proteasome regulatory particle assembly. Nature, 459 (7248): 861–865

Russell S J, Steger K A, Johnston S A (1999). Subcellular localization, stoichiometry, and protein levels of 26 S proteasome subunits in yeast. J Biol Chem, 274(31): 21943–21952

Sa-Moura B, Simões A M, Fraga J, Fernandes H, Abreu I A, Botelho H M, Gomes C M, Marques A J, Dohmen R J, Ramos P C, Macedo-Ribeiro S (2013). Biochemical and biophysical characterization of recombinant yeast proteasome maturation factor ump1. Comput Struct Biotechnol J, 7(8): e201304006

Sadre-Bazzaz K, Whitby F G, Robinson H, Formosa T, Hill C P (2010). Structure of a Blm10 complex reveals common mechanisms for proteasome binding and gate opening. Mol Cell, 37(5): 728–735

Saeki Y, Toh E A, Kudo T, Kawamura H, Tanaka K (2009). Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle. Cell, 137(5): 900–913

Sakata E, Stengel F, Fukunaga K, Zhou M, Saeki Y, Förster F, Baumeister W, Tanaka K, Robinson C V (2011). The catalytic activity of Ubp6 enhances maturation of the proteasomal regulatory particle. Mol Cell, 42(5): 637–649

Satoh T, Saeki Y, Hiromoto T, Wang Y H, Uekusa Y, Yagi H, Yoshihara H, Yagi-Utsumi M, Mizushima T, Tanaka K, Kato K (2014). Structural basis for proteasome formation controlled by an assembly chaperone nas2. Structure, 22(5): 731–743

Savulescu A F, Shorer H, Kleifeld O, Cohen I, Gruber R, Glickman M H, Harel A (2011). Nuclear import of an intact preassembled proteasome particle. Mol Biol Cell, 22(6): 880–891

Schmidt M, Haas W, Crosas B, Santamaria P G, Gygi S P, Walz T, Finley D (2005). The HEAT repeat protein Blm10 regulates the yeast proteasome by capping the core particle. Nat Struct Mol Biol, 12(4): 294–303

Schmidtke G, Kraft R, Kostka S, Henklein P, Frömmel C, Löwe J, Huber R, Kloetzel P M, Schmidt M (1996). Analysis of mammalian 20S proteasome biogenesis: the maturation of beta-subunits is an ordered two-step mechanism involving autocatalysis. EMBO J, 15: 6887–6898

Schmidtke G, Schmidt M, Kloetzel P M (1997). Maturation of mammalian 20 S proteasome: purification and characterization of 13 S and 16 S proteasome precursor complexes. J Mol Biol, 268(1): 95–106

Schweitzer A, Aufderheide A, Rudack T, Beck F, Pfeifer G, Plitzko J M, Sakata E, Schulten K, Förster F, Baumeister W (2016). Structure of the human 26S proteasome at a resolution of 3.9 A. Proc Natl Acad Sci USA, 113(28): 7816–7821

Sha Z, Goldberg A L (2014). Proteasome-mediated processing of Nrf1 is essential for coordinate induction of all proteasome subunits and p97. Curr Biol, 24(14): 1573–1583

Sharon M, Taverner T, Ambroggio X I, Deshaies R J, Robinson C V (2006). Structural organization of the 19S proteasome lid: insights from MS of intact complexes. PLoS Biol, 4(8): e267

Sharon M, Witt S, Glasmacher E, Baumeister W, Robinson C V (2007). Mass spectrometry reveals the missing links in the assembly pathway of the bacterial 20 S proteasome. J Biol Chem, 282(25): 18448–18457

Shi Y, Chen X, Elsasser S, Stocks B B, Tian G, Lee B H, Shi Y, Zhang N, de Poot S A H, Tuebing F, Sun S, Vannoy J, Tarasov S G, Engen J R, Finley D, Walters K J (2016). Rpn1 provides adjacent receptor sites for substrate binding and deubiquitination by the proteasome. Science

Shirozu R, Yashiroda H, Murata S (2015). Identification of minimum Rpn4-responsive elements in genes related to proteasome functions. FEBS Lett, 589(8): 933–940

Singh C R, Lovell S, Mehzabeen N, Chowdhury W Q, Geanes E S, Battaile K P, Roelofs J (2014). 1.15 A resolution structure of the proteasome-assembly chaperone Nas2 PDZ domain. Acta Crystallogr F Struct Biol Commun, 70(4): 418–423

Sledz P, Unverdorben P, Beck F, Pfeifer G, Schweitzer A, Forster F, Baumeister W (2013). Structure of the 26S proteasome with ATP-gammaS bound provides insights into the mechanism of nucleotide-dependent substrate translocation. Proc Natl Acad Sci USA, 110(18): 7264–7269

Smith D M, Chang S C, Park S, Finley D, Cheng Y, Goldberg A L (2007). Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's alpha ring opens the gate for substrate entry. Mol Cell, 27(5): 731–744

Sokolova V, Li F, Polovin G, Park S (2015). Proteasome activation is mediated via a functional switch of the Rpt6 C-terminal tail following chaperone-dependent assembly. Sci Rep, 5: 14909

Stadtmueller B M, Hill C P (2011). Proteasome activators. Mol Cell, 41 (1): 8–19

Stadtmueller B M, Kish-Trier E, Ferrell K, Petersen C N, Robinson H, Myszka D G, Eckert D M, Formosa T, Hill C P (2012). Structure of a proteasome Pba1-Pba2 complex: implications for proteasome assembly, activation, and biological function. J Biol Chem, 287 (44): 37371–37382

Takagi K, Kim S, Yukii H, Ueno M, Morishita R, Endo Y, Kato K, Tanaka K, Saeki Y, Mizushima T (2012). Structural basis for specific recognition of Rpt1p, an ATPase subunit of 26S proteasome, by proteasome-dedicated chaperone Hsm3p. J Biol Chem, 287(15): 12172–12182

Takagi K, Saeki Y, Yashiroda H, Yagi H, Kaiho A, Murata S, Yamane T, Tanaka K, Mizushima T, Kato K (2014). Pba3-Pba4 heterodimer acts as a molecular matchmaker in proteasome alpha-ring formation. Biochem Biophys Res Commun, 450(2): 1110–1114

Takeuchi J, Tamura T (2004). Recombinant ATPases of the yeast 26S proteasome activate protein degradation by the 20S proteasome. FEBS Lett, 565(1-3): 39–42

Tanaka K, Yoshimura T, Tamura T, Fujiwara T, Kumatori A, Ichihara A (1990). Possible mechanism of nuclear translocation of proteasomes. FEBS Lett, 271(1-2): 41–46

Thompson D, Hakala K, DeMartino G N (2009). Subcomplexes of PA700, the 19S regulator of the 26 S proteasome, reveal relative roles of AAA subunits in 26 S proteasome assembly and activation and ATPase activity. J Biol Chem, 284(37): 24891–24903

Tian G, Park S, Lee M J, Huck B, McAllister F, Hill C P, Gygi S P, Finley D (2011). An asymmetric interface between the regulatory and core particles of the proteasome. Nat Struct Mol Biol, 18(11): 1259–1267

Tomko R J Jr, Funakoshi M, Schneider K, Wang J, Hochstrasser M (2010). Heterohexameric ring arrangement of the eukaryotic proteasomal ATPases: implications for proteasome structure and assembly. Mol Cell, 38(3): 393–403

Tomko R J Jr, Hochstrasser M (2011). Incorporation of the Rpn12

subunit couples completion of proteasome regulatory particle lid assembly to lid-base joining. Mol Cell, 44(6): 907–917

Tomko R J Jr, Hochstrasser M (2013). Molecular architecture and assembly of the eukaryotic proteasome. Annu Rev Biochem, 82(1): 415–445

Tomko R J Jr, Hochstrasser M (2014). The intrinsically disordered Sem1 protein functions as a molecular tether during proteasome lid biogenesis. Mol Cell, 53(3): 433–443

Tomko R J Jr, Taylor D W, Chen Z A, Wang H W, Rappsilber J, Hochstrasser M (2015). A Single alpha helix drives extensive remodeling of the proteasome lid and completion of regulatory particle assembly. Cell, 163(2): 432–444

Uekusa Y, Okawa K, Yagi-Utsumi M, Serve O, Nakagawa Y, Mizushima T, Yagi H, Saeki Y, Tanaka K, Kato K (2014). Backbone (1)H, (1)(3)C and (1)(5)N assignments of yeast Ump1, an intrinsically disordered protein that functions as a proteasome assembly chaperone. Biomol NMR Assign, 8(2): 383–386

Unno M, Mizushima T, Morimoto Y, Tomisugi Y, Tanaka K, Yasuoka N, Tsukihara T (2002). The structure of the mammalian 20S proteasome at 2.75 A resolution. Structure, 10(5): 609–618

Unverdorben P, Beck F, led P, Schweitzer A, Pfeifer G, Plitzko J M, Baumeister W, Forster F (2014). Deep classification of a large cryo-EM dataset defines the conformational landscape of the 26S proteasome. Proc Natl Acad Sci USA, 111(15): 5544–5549

Ustrell V, Hoffman L, Pratt G, Rechsteiner M (2002). PA200, a nuclear proteasome activator involved in DNA repair. EMBO J, 21(13): 3516–3525

Velichutina I, Connerly P L, Arendt C S, Li X, Hochstrasser M (2004). Plasticity in eucaryotic 20S proteasome ring assembly revealed by a subunit deletion in yeast. EMBO J, 23(3): 500–510

Verma R, et al (2002). Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. Science, 298(5593): 611–615

Volker C, Lupas A N (2002). Molecular evolution of proteasomes. Curr Top Microbiol Immunol, 268: 1–22

Waite K A, De-La Mota-Peynado A, Vontz G, Roelofs J (2016). Starvation induces proteasome autophagy with different pathways for core and regulatory particles. J Biol Chem, 291(7): 3239–3253

Wang H R, Kania M, Baumeister W, Nederlof P M (1997). Import of human and Thermoplasma 20S proteasomes into nuclei of HeLa cells requires functional NLS sequences. Eur J Cell Biol, 73: 105–113

Wang W, Chan J Y (2006). Nrf1 is targeted to the endoplasmic reticulum membrane by an N-terminal transmembrane domain. Inhibition of nuclear translocation and transacting function. J Biol Chem, 281(28): 19676–19687

Wani P S, Rowland M A, Ondracek A, Deeds E J, Roelofs J (2015). Maturation of the proteasome core particle induces an affinity switch that controls regulatory particle association. Nat Commun, 6: 6384

Wani P S, Suppahia A, Capalla X, Ondracek A, Roelofs J (2016). Phosphorylation of the C-terminal tail of proteasome subunit alpha7 is required for binding of the proteasome quality control factor Ecm29. Sci Rep, 6: 27873

Weberruss M H, Savulescu A F, Jando J, Bissinger T, Harel A, Glickman M H, Enenkel C (2013). Blm10 facilitates nuclear import of proteasome core particles. EMBO J, 32(20): 2697–2707

Wei S J, Williams J G, Dang H, Darden T A, Betz B L, Humble M M, Chang F M, Trempus C S, Johnson K, Cannon R E, Tennant R W (2008). Identification of a specific motif of the DSS1 protein required for proteasome interaction and p53 protein degradation. J Mol Biol, 383(3): 693–712

Welk V, Coux O, Kleene V, Abeza C, Trümbach D, Eickelberg O, Meiners S (2016). Inhibition of proteasome activity induces formation of alternative proteasome complexes. J Biol Chem, 291 (25): 13147–13159

Wendler P, Lehmann A, Janek K, Baumgart S, Enenkel C (2004). The bipartite nuclear localization sequence of Rpn2 is required for nuclear import of proteasomal base complexes via karyopherin alphabeta and proteasome functions. J Biol Chem, 279(36): 37751–37762

Whitby F G, Masters E I, Kramer L, Knowlton J R, Yao Y, Wang C C, Hill C P (2000). Structural basis for the activation of 20S proteasomes by 11S regulators. Nature, 408(6808): 115–120

Witt E, Zantopf D, Schmidt M, Kraft R, Kloetzel P M, Kruger E (2000). Characterisation of the newly identified human Ump1 homologue POMP and analysis of LMP7(beta 5i) incorporation into 20 S proteasomes. J Mol Biol, 301(1): 1–9

Wollenberg K, Swaffield J C (2001). Evolution of proteasomal ATPases. Mol Biol Evol, 18(6): 962–974

Worden E J, Padovani C, Martin A (2014). Structure of the Rpn11-Rpn8 dimer reveals mechanisms of substrate deubiquitination during proteasomal degradation. Nat Struct Mol Biol, 21(3): 220–227

Xie Y, Varshavsky A (2001). RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. Proc Natl Acad Sci USA, 98(6): 3056–3061

Yao T, Cohen R E (2002). A cryptic protease couples deubiquitination and degradation by the proteasome. Nature, 419(6905): 403–407

Yao Y, Toth C R, Huang L, Wong M L, Dias P, Burlingame A L, Coffino P, Wang C C (1999). alpha5 subunit in *Trypanosoma brucei* proteasome can self-assemble to form a cylinder of four stacked heptamer rings. Biochem J, 344(Pt 2): 349–358

Yashiroda H, Mizushima T, Okamoto K, Kameyama T, Hayashi H, Kishimoto T, Niwa S, Kasahara M, Kurimoto E, Sakata E, Takagi K, Suzuki A, Hirano Y, Murata S, Kato K, Yamane T, Tanaka K (2008). Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes. Nat Struct Mol Biol, 15(3): 228–236

Yashiroda H, Toda Y, Otsu S, Takagi K, Mizushima T, Murata S (2015). N-terminal alpha7 deletion of the proteasome 20S core particle substitutes for yeast PI31 function. Mol Cell Biol, 35(1): 141–152

Yu Y, Smith D M, Kim H M, Rodriguez V, Goldberg A L, Cheng Y (2010). Interactions of PAN's C-termini with archaeal 20S proteasome and implications for the eukaryotic proteasome-ATPase interactions. EMBO J, 29(3): 692–702

Yu Z, Livnat-Levanon N, Kleifeld O, Mansour W, Nakasone M A, Castaneda C A, Dixon E K, Fushman D, Reis N, Pick E, Glickman M H (2015). Base-CP proteasome can serve as a platform for stepwise lid formation. Biosci Rep, 35(3): e00194

Zaiss D M, Standera S, Kloetzel P M, Sijts A J (2002). PI31 is a modulator of proteasome formation and antigen processing. Proc Natl Acad Sci USA, 99(22): 14344–14349

Zhang F, Hu M, Tian G, Zhang P, Finley D, Jeffrey P D, Shi Y (2009). Structural insights into the regulatory particle of the proteasome from Methanocaldococcus jannaschii. Mol Cell, 34(4): 473–484

Zhang Y, Lucocq J M, Yamamoto M, Hayes J D (2007). The NHB1 (N-terminal homology box 1) sequence in transcription factor Nrf1 is

required to anchor it to the endoplasmic reticulum and also to enable its asparagine-glycosylation. Biochem J, 408(2): 161–172

Zhu K, Dunner K Jr, McConkey D J (2010). Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene, 29(3): 451–462

Zuhl F, Seemuller E, Golbik R, Baumeister W (1997). Dissecting the assembly pathway of the 20S proteasome. FEBS Lett, 418(1-2): 189–194

Zwickl P, Kleinz J, Baumeister W (1994). Critical elements in proteasome assembly. Nat Struct Biol, 1(11): 765–770

Zwickl P, Ng D, Woo K M, Klenk H P, Goldberg A L (1999). An archaebacterial ATPase, homologous to ATPases in the eukaryotic 26S proteasome, activates protein breakdown by 20 S proteasomes. J Biol Chem, 274(37): 26008–26014