Proteasome assembly: **Biting the hand...**

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Proteasome assembly is regulated to ensure the enzyme is inactive until its active sites are compartmentalized within an interior aqueous chamber. In yeast, this depends on a dedicated chaperone that is trapped within the nascent proteasome, and degraded on maturation of the proteolytic subunits.

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Molecular chaperones are a class of proteins whose definition is evolving as rapidly as new varieties are being discovered. In the most general sense, molecular chaperones are proteins that engage in reversible binding interactions with other proteins, and thereby influence the rate and efficiency of folding, unfolding, assembly and disassembly pathways. In recent years, we have become aware of a remarkable variety of proteins that display chaperone-like functions and a multiplicity of pathways that are affected by, and even dependent on, those functions. Once the province of two families of proteins, the heat-shock protein 70 (Hsp70) chaperones and Hsp60 chaperonins, chaperoning functions have now been described for nearly a dozen different classes of proteins, as well as for an unknown but certainly large number of 'dedicated' chaperone-like proteins. The latest — one is tempted to say late — member of the dedicated chaperone class is the yeast protein Ump1p [1]. Ump1p helps the formation of functional yeast 20 S proteasomes, and winds up being degraded for its efforts.

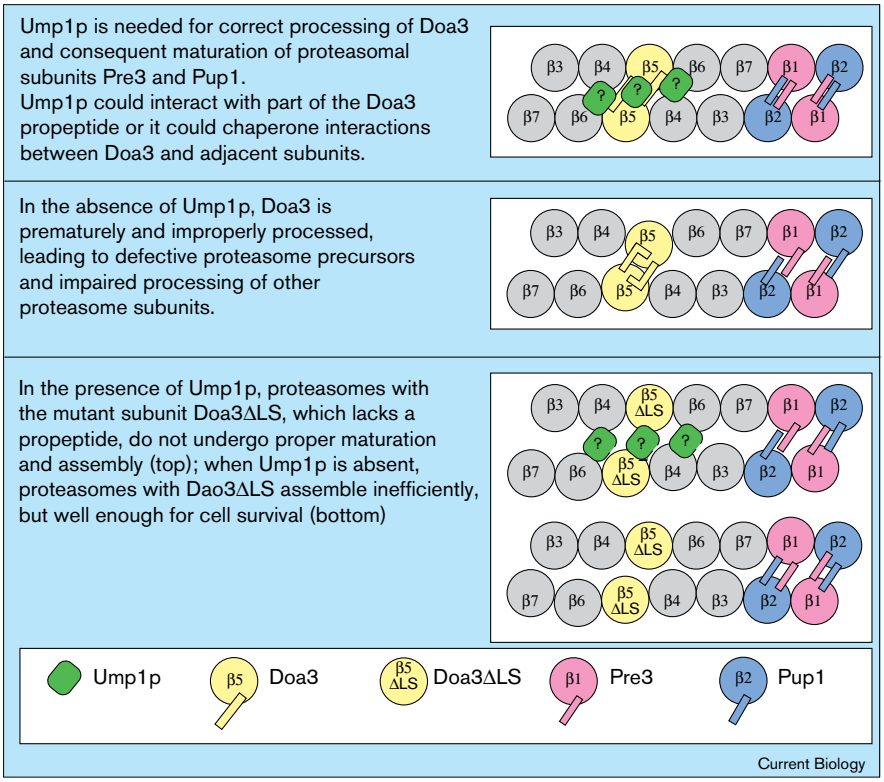
Ramos *et al.* [1] identified Ump1p by the ability of its gene to complement a *ump1* mutant yeast strain, which had been identified by its defect in degradation of short-lived proteins. Ump1p was subsequently shown to bind to a precursor of the 20 S proteasome, become enclosed within the particle when two half-proteasomes join, and be degraded following activation of the catalytic subunits. As well as physically filling the gap between nascent proteasome rings, Ump1p also bridges a figurative second gap — that between intramolecular chaperones, such as the pro-regions of various proteases, which aid in folding and are subsequently cleaved off and degraded, and extra-molecular chaperones, such as Hsp70s, which undergo cycles of folding and unfolding seemingly without limit.

Why does Ump1p have such a short life span? Is it an accident of fate — did it simply get assigned to chaperone a dance in the wrong part of town? Or rather, is its short-lived role another demonstration of the care with which the cell assembles and starts in motion the lethal machinery of protein degradation — is Ump1p the sacrificial lamb that appeases the degradative demon in the machine, while assuring that the proteolytic function is confined before it is turned on? Everything we have learned about the multiple layers of control over protein degradation favors the latter view.

The 20 S proteasome is the major cytosolic protease in eukaryotic cells and is the proteolytic component of the ubiquitin-dependent degradative pathway [2]. Proteasomes are also found in some, but not all, archaebacteria and eubacteria. True proteasomes are composed of 28 subunits, 14 each of two different classes, the non-catalytic alpha and catalytically-active beta subunits. The subunits are arranged in rings of seven subunits, all of a single type. The 20 S proteasome is a stack of four rings, two inner beta rings flanked by the alpha rings. The junction between the beta rings produces a remarkable structural feature of proteasomes — an interior aqueous cavity large enough to accommodate about 70 kDa of protein and accessible only through narrow axial channels in the rings [3,4]. The catalytic sites are located on the beta subunits within the aqueous cavity. Isolation of the catalytic sites in this way, and the limited access via narrow channels, serves to compartmentalize proteolysis [2], allowing degradation of only those proteins that can be actively translocated into the interior of the proteasome.

Assembling the eukaryotic 20 S proteasome is a daunting challenge, and not surprisingly the system gets outside help from at least one, and possibly other, chaperones. Unlike the prototypical proteasome from the bacterium *Thermoplasma*, which has just one type each of alpha and beta subunit, eukaryotic proteasomes have seven different types of alpha and beta subunit. Other organisms have different numbers of alpha and beta subunits in their proteasomes. Assembly of the eukaryotic proteasome is uniquely difficult, because each alpha or beta ring bears one each of the seven different subunits, which are arranged in an ordered sequence around the ring [3]. In bacterial proteasomes, all the beta subunits are catalytically active, so there are 14 active sites (one per subunit) within the core. In contrast, only three of the seven eukaryotic beta subunits have the necessary catalytic residues, so there are only six active sites within the degradative chamber. Within each ring, the catalytic subunits are positioned

Figure 1



The central proteasome beta-subunit rings are shown unwound and stacked as they occur in the yeast proteasome. Left to right on top corresponds to clockwise rotation looking along the cylindrical axis from the center of the particle. Only the proteolytically active subunits are identified by colors and specific names, and propeptides are shown for those subunits. The altered conformation of the Doa3 propeptide is implied from the incorrect processing that occurs in the absence of Ump1p.

such that two lie adjacent to each other and the third is opposite them, separated by two non-catalytic subunits on each side (Figure 1).

The alpha and beta subunits evolved from a common ancestor and have very similar secondary and tertiary structures. Alpha and beta subunits can segregate faithfully into the separate rings, in part because of a considerable divergence in their amino-terminal sequences. The crystal structures show that amino terminal extensions form part of the subunit bonding domains between alpha subunits. Alpha subunits of the *Thermoplasma* proteasome oligomerize spontaneously *in vitro* [5], and human proteasomal alpha subunit HsC8 will also assemble into rings [6]. Two other human alpha subunits were found to form dimers; they failed to oligomerize further by themselves, but could be incorporated into ring structures when HsC8 was added.

Beta subunits do not oligomerize spontaneously, but will readily assemble in the presence of a complement of alpha subunits. The amino-terminal extensions of beta subunits are highly divergent and contain proregions that are autocatalytically removed in the mature proteasome. The proregions of several eukaryotic beta subunits are quite long (60–80 amino acids) and play critical roles in assembly and maturation of the proteasome. They may not, however, be sufficient as targeting signals for positioning of subunits, as a chimera of human beta subunit LMP7 carrying the propeptide from LMP2 was found to occupy the normal position for LMP7 in the ring [7]. The altered propeptide did, however, affect the efficiency of cleavage of the propeptide; about two-thirds of the chimeric molecules were processed correctly but the remainder retained nine additional amino acids.

Proteasome assembly *in vivo* is relatively slow, and a significant fraction of proteasome subunits can be isolated in precursor form. The smallest precursor complex isolated (13 S) contains all seven alpha subunits and probably three unprocessed beta subunits [8]. How this species arises is not known. The eukaryotic pathway is perhaps a hybrid of the bacterial ones. *Thermoplasma* alpha rings act as a template for assembly of the beta subunits, whereas the *Rhodococcus* alpha and beta subunits form heterodimers which then assemble into rings [5,9]. The second intermediate on the eukaryotic pathway appears to be a 'half-proteasome' (15–16 S) consisting of a complex between an alpha and a beta ring. The half-proteasome contains mostly unprocessed beta subunits, although there are data suggesting that some degree of processing is possible in this state. Half-proteasomes tend to be shorter-lived than the 13 S precursor, indicating that once the last beta subunits are incorporated, the final assembly and maturation steps proceed

more efficiently. In yeast, mutations that disrupt interactions between half-proteasomes interfere with processing of proteasomal subunits and subsequent assembly [10].

The lack of prosequence processing activity in half-proteasomes raised the possibility that processing occurs across the dyad symmetry axis after the intact proteasome is formed [10]. Alternatively, contacts between the half-proteasomes could alter the positions of the propeptides or the conformation of the catalytic sites thereby facilitating autocatalytic cleavage within a ring. Studies addressing these issues in the yeast system have shed light on the timing of propeptide cleavage and on the role of the propeptide in maturation of the proteasome. Now, approaching the problem from a very different direction, Ramos *et al.* [1] have shown that at least one cellular protein is cast in the role of a dedicated chaperone for this process.

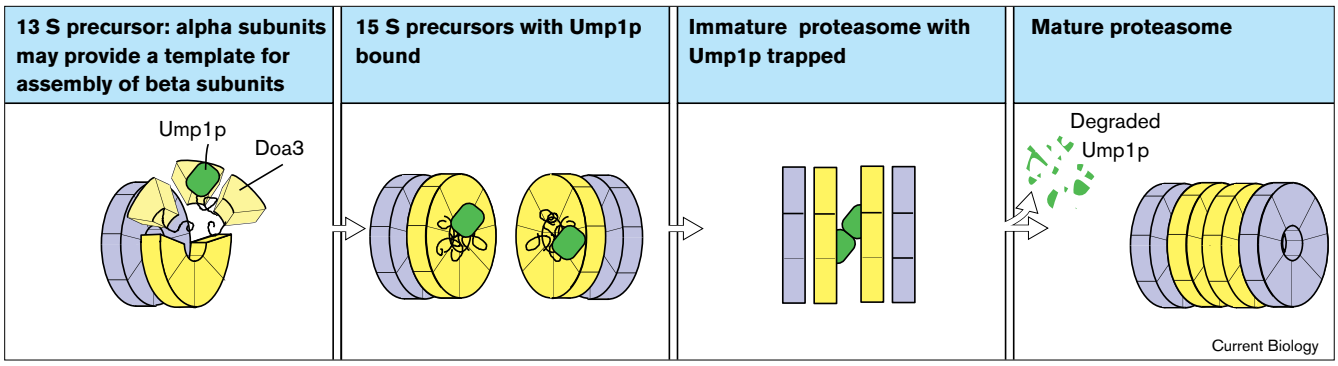
Proteasome propeptides, by analogy with propeptides from other proteases [11], could function in two ways. They could block the catalytic activity of the subunit until the active sites are safely isolated within the degradative chamber, or they could contribute to subunit folding and stability during assembly. A mutant form of the beta subunit Doa3, Doa3ΔLS, which lacks a propeptide, was not incorporated efficiently into yeast proteasomes, resulting in the formation of defective proteasomes. Moreover, expression of the Doa3 propeptide in *trans* allowed incorporation of Doa3ΔLS into proteasomes with high efficiency [10], suggesting an active role for the propeptide.

It was not, however, possible from these experiments to conclude whether the Doa3 propeptide was needed to promote the folding and uptake into the proteasome of Doa3 or other subunits, or to prevent premature expression of the chymotrypsin-like activity of Doa3. To discriminate between the possibilities, Chen and Hochstrasser [10] generated a mutation in a catalytic residue of Doa3ΔLS which eliminated its chymotrypsin-like activity. The mutant strain grew well only when the propeptide was supplied in *trans*. In the presence of the propeptide, the mutant strain was viable and proteasome assembly was normal, although the proteasomes now lacked chymotrypsin-like activity and the substrate, MATα2 repressor, was stabilized about sixfold. Thus, the Doa3 propeptide has a role beyond preventing premature expression of chymotrypsin-like activity, suggesting that it might act as a chaperone in proteasome assembly.

Ramos *et al.* [1] became interested in the yeast *UMP1* gene because *ump1* mutant cells are defective in the degradation of several proteins targeted by the ubiquitin system, and it appeared that the gene product acts downstream of the ubiquitin pathway. After isolation of the wild-type gene, Ump1 protein was expressed with an epitope tag, and cell extracts were analyzed to locate the protein. It became apparent that Ump1p fractionated with 15 S proteasome precursors. When proteasomal subunits were examined, all three of the catalytic subunits — Pup1, Pre3 and Doa3 — had severe defects in processing to their mature proteasomal form. When enzymatic activities of these subunits were measured, Pup1 had very low trypsin-like activity and Pre3 had decreased peptidylglutamyl peptide-hydrolyzing activity, but high levels of chymotrypsin-like activity, normally associated with Doa3, were found. The latter activity was expressed in particles that sedimented as incomplete proteasomes. This led Ramos *et al.* [1] to speculate that Ump1p may be a proteasome-specific chaperone that is required for proper timing of precursor processing.

Ump1p was not found in 20 S proteasomes. Pulse-chase experiments showed that the protein had a half-life of 20 minutes, similar to the half-time of processing of

Figure 2



Assembly of the proteasome occurs via a 13 S precursor that includes all of the alpha subunits (blue) and probably four of the beta subunits (yellow). Ump1p is shown binding to Doa3, but no direct evidence for this interaction has been published. Ump1p remains bound and is trapped in the central degradative chamber after assembly of the 20 S proteasome, where it is degraded.

proteasomal subunits. Ramos *et al.* [1] then examined *pre1-1* mutant cells, which have lost the chymotrypsin-like activity of Doa3 because of defective subunit interactions, and found that Ump1p could be isolated with 20 S proteasomes. The proteasome-bound Ump1p was not accessible to Ump1p-specific antibodies unless the complex was denatured in detergent, and it was resistant to trypsin. Ump1p bound to wild-type half-proteasomes was accessible to both antibodies and exogenous protease. Thus, Ump1p appears to be contained within the mutant proteasome. A model based on these [1] and other [8] data is illustrated in Figure 2.

It may come as no surprise that a complex like the proteasome would take advantage of cellular chaperones in its assembly. In humans, two different sets of the three catalytic subunits are incorporated in normal or γ-interferon-induced cells, producing functionally distinct proteasomes, which presents unique sorting and assembly problems. Low molecular weight proteins, possibly similar to Ump1p, have been found with mammalian proteasome precursors [7,8], suggesting that such dedicated chaperones might be important in the assembly of all complex proteasomes.

Several aspects of the Ump1p story are especially intriguing. Firstly, further experiments by Ramos *et al.* [1] unveiled a head-on crash between Ump1p and the other novel chaperone in the proteasome assembly story — the propeptide of Doa3. It turns out that *ump1* mutants no longer require the Doa3 propeptide to grow. The double mutant *ump1 doa3ΔLS* was found to be viable, but failed to grow if Ump1p was supplied from an inducible expression system. Although the double mutant made partially defective proteasomes, these results suggest that the site of action of Ump1p and the Doa3 propeptide may overlap, or that the proteins may have some activities that are mutually antagonistic.

The second intriguing point is that Ump1p has some short regions of sequence similarity with contrapsin and other protease inhibitors. If Ump1p binds at a nascent proteolytic active site and prevents its premature activation, does that make it a chaperone? Are propeptides of other proteases molecular chaperones? Propeptides are thought to provide a template for folding of the mature protease [11] — mutations in the propeptide region can alter the final conformation of the protease! Is it useful to label every ligand that promotes a particular conformation or alters a folding pathway a chaperone? It might be more useful to distinguish between a protein that acts as a template for another's folding or binds specifically to a unique site thus favoring a particular folding pathway, and the energy-dependent molecular chaperones, which interact repeatedly with unfolded proteins. Chaperones must process information concerning the native structure of proteins, and that information comes at a high cost in ATP consumed.

Lastly, Ump1p may be the first *bona fide* substrate for the 20 S proteasome that does not require a regulatory component. Or does it? The experiments described by Ramos *et al.* [1] do not indicate whether Ump1p is degraded spontaneously upon joining of the two half-proteasomes and processing of Doa3. Ump1p is found in mutant proteasome species that sediment faster than 20 S; it is possible that a further, perhaps final, activation step involving the 19 S or another regulator is required. In any event, this study has ushered in yet another new chapter in the seemingly never-ending tale of the proteasome and ubiquitin-mediated proteolysis.

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