Check for updates

REVIEW

Intracellular Dynamics of the Ubiquitin-Proteasome-System

[version 1; peer review: 3 approved]

Maisha Chowdhury, Cordula Enenkel

Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Canada

V1 First published: 24 Jul 2015, 4:367
<https://doi.org/10.12688/f1000research.6835.1>
Latest published: 28 Sep 2015, 4:367
<https://doi.org/10.12688/f1000research.6835.2>

Abstract

The ubiquitin-proteasome system is the major degradation pathway for short-lived proteins in eukaryotic cells. Targets of the ubiquitin-proteasome-system are proteins regulating a broad range of cellular processes including cell cycle progression, gene expression, the quality control of proteostasis and the response to geno- and proteotoxic stress. Prior to degradation, the proteasomal substrate is marked with a poly-ubiquitin chain. The key protease of the ubiquitin system is the proteasome. In dividing cells, proteasomes exist as holo-enzymes composed of regulatory and core particles. The regulatory complex confers ubiquitin-recognition and ATP dependence on proteasomal protein degradation. The catalytic sites are located in the proteasome core particle. Proteasome holo-enzymes are predominantly nuclear suggesting a major requirement for proteasomal proteolysis in the nucleus. In cell cycle arrested mammalian or quiescent yeast cells, proteasomes deplete from the nucleus and accumulate in granules at the nuclear envelope (NE) / endoplasmic reticulum (ER) membranes. In prolonged quiescence, proteasome granules drop off the NE / ER membranes and migrate as stable organelles throughout the cytoplasm, as thoroughly investigated in yeast. When quiescence yeast cells are allowed to resume growth, proteasome granules clear and proteasomes are rapidly imported into the nucleus.

Here, we summarize our knowledge about the enigmatic structure of proteasome storage granules and the trafficking of proteasomes and their substrates between the cyto- and nucleoplasm.

Most of our current knowledge is based on studies in yeast. Their translation to mammalian cells promises to provide keen insight into protein degradation in non-dividing cells which comprise the majority of our body's cells.

Keywords

Proteasome, Storage Granules, Dynamics, Nuclear Transport, Ubiquitin System, Blm10, Importin, Karyopherin, Quiescence

Open Peer Review

Approval Status

	1	2	3
version 2 (revision) 28 Sep 2015			
version 1 24 Jul 2015	 view	 view	 view

1. **Petra Wendler**, Ludwig Maximilian University of Munich, Munich, Germany
2. **Philip Coffino**, University of California, San Francisco, San Francisco, USA
3. **Paula C. Ramos**, University of Cologne, Cologne, Germany

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Cordula Enenkel (cordula.enenkel@utoronto.ca)

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by grants from NSERC (4422666-2011) awarded to M.C. and C.E. and from CIHR (325477) awarded to C.E.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2015 Chowdhury M and Enenkel C. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Chowdhury M and Enenkel C. **Intracellular Dynamics of the Ubiquitin-Proteasome-System [version 1; peer review: 3 approved]** F1000Research 2015, **4**:367 <https://doi.org/10.12688/f1000research.6835.1>

First published: 24 Jul 2015, **4**:367 <https://doi.org/10.12688/f1000research.6835.1>

Introduction

Proteolysis determines the half-life of proteins and thus controls protein homeostasis. If protein homeostasis is disrupted, the incidence of protein misfolding and neurodegenerative diseases such as Huntington's, Parkinson's and Alzheimer's increases (Ciechanover & Brundin, 2003).

In eukaryotic cells two highly conserved degradation pathways exist: long-lived proteins are degraded within the lysosome, an organelle with membranes which protect the surrounding cytoplasm against lysosomal hydrolases (Rendueles & Wolf, 1988); short-lived proteins are degraded by proteasomes, multimeric protease complexes which move between the nucleolar and cytoplasm (Hershko & Ciechanover, 1998). Proteasomal substrates are often nuclear proteins such as proteins regulating cell cycle progression (cyclin-dependant kinases and their inhibitors), gene expression (transcription factors), DNA damage and stress response; although, misfolded proteins occurring during protein synthesis in the cytoplasm are also rapidly degraded by the proteasome (Kirschner, 1999; Vabulas & Hartl, 2005; von Mikecz, 2006). As a result proteasomal proteolysis serves to eliminate obsolete proteins which compete with functional proteins for binding partners and are prone to associate with irreversible and toxic protein aggregates (Goldberg, 2003).

Here, we want to address the dynamics of proteasomes, which select their substrates by specific determinants such as poly-ubiquitylation, a covalently linked chain of ubiquitin molecules (Finley, 2009). This ubiquitin-dependent proteolysis undertakes up to 90% of protein degradation in growing yeast and cultured mammalian cells and consumes considerable amounts of ATP, since the activation and conjugation of ubiquitin to the protein substrate as well as the unfolding and translocation of the protein substrate into the proteasome is ATP-dependent (Coux *et al.*, 1996). Natively-disordered proteins also qualify as proteasome substrates and are cleaved without post-translational ubiquitin modification (Fishbain *et al.*, 2015).

The advent of live cell imaging and GFP-labelling technologies in the 1990s (Tsien, 1998) have greatly facilitated the study of proteasome dynamics in yeast and mammalian cells. Through these non-invasive techniques, the localization of the proteasome in growing yeast and highly proliferating cancer cells has been elucidated to be primarily nuclear (Enenkel *et al.*, 1998; Laporte *et al.*, 2008; McDonald & Byers, 1997; Russell *et al.*, 1999). In line with this finding, increasing evidence in the literature suggests that certain misfolded proteins are imported from the cytoplasm into the nucleus solely for proteasomal degradation (Park *et al.*, 2013; Prasad *et al.*, 2010). Conversely, transient nuclear proteins are exported into the cytoplasm for proteolysis, indicating a dynamic shift of proteasomal substrates between the nucleus and cytoplasm (Chen & Madura, 2014a). Under nutrient deprivation and during transition from proliferation to quiescence, yeast proteasomes gather in proteasome storage granules (PSGs) at the nuclear envelope (NE) and endoplasmic reticulum (ER) membrane (Enenkel, 2014; Knecht & Rivett, 2000; Wojcik & DeMartino, 2003). PSGs are membraneless organelles which seem to pinch off the NE/ER and are retained as

stable entities in the cytoplasm. When cells resume growth, PSGs dissipate and proteasomes are rapidly imported into the nucleus to contribute their function in cell proliferation (Laporte *et al.*, 2008). The mechanism of PSG formation and clearance is still unknown but seems to be conserved, since PSG-like structures are observed in primary cell lines of non-dividing neuronal cells and in immortalized cell lines of cancer cells, if they are chemically arrested in cell cycle progression (Kaganovich *et al.*, 2008).

Our knowledge about proteasome dynamics in mammalian cells is poor. Thus, the focus of this review will be to critically integrate the literature about the dynamics of the proteasome, particularly based on studies in yeast. In our overview of the ubiquitin-proteasome system and common principles of nuclear transport, we cite and refer to original work and review articles written by investigators who did seminal work on these topics. In the paragraphs addressing detailed knowledge about proteasome dynamics we cite the original work.

Discussion/analysis of the literature

The ubiquitin system

Ubiquitylation is a post-translational modification commonly associated with proteasomal protein degradation. At least four ubiquitin molecules, conserved peptides of 76 amino acids, are required for a poly-ubiquitin chain to be recognized by the proteasome. Hershko and colleagues in the early 1980s showed that poly-ubiquitylation requires three ATP-dependent enzymes: the ubiquitin activation enzyme (E1), a family of ubiquitin conjugating enzymes (E2) and a family of ubiquitin protein ligases (E3) (Hershko & Ciechanover, 1998). First, ATP hydrolysis is required to activate the AMP linkage to the C-terminal glycine of ubiquitin which enables the transfer of the ubiquitin moiety to the active site cysteine of the E1. Second, the E1-bound ubiquitin is linked to the active site cysteine residue of an E2 by transesterification. Finally, the E3 transfers the ubiquitin onto the substrate depending on the class of the E3 enzyme (RING, HECT and U-box ligases) (Finley *et al.*, 2012; Harper & Schulman, 2006). Upon transfer of ubiquitin onto the substrate, the C-terminal glycine forms an isopeptide bond with an ϵ -amino group of a lysine residue in the substrate. Elongation of the ubiquitin chain is achieved as succeeding ubiquitin form isopeptide linkages with specific lysines of the preceding ubiquitin (Hershko & Ciechanover, 1998). Prior to degradation, deubiquitinating activities within the proteasome cleave and recycle the ubiquitin molecules from the substrates (Crosas *et al.*, 2006; Hanna *et al.*, 2006; Lam *et al.*, 1997; Verma *et al.*, 2002). Deubiquitinating enzymes in the cyto- and nucleoplasm provide an additional level on the plasticity on the repertoire of proteasomal substrates (Sahtoe & Sixma, 2015). Intriguingly, GFP-labelled ubiquitin and the E1, named UBA1, is primarily nuclear in growing yeast and mammalian cells suggesting that ubiquitin-dependent proteolysis mainly occurs in the nucleus (Huh *et al.*, 2003; Salomons *et al.*, 2010; Sugaya *et al.*, 2014; Sugaya *et al.*, 2015).

Proteasome assembly and composition

Composed of over 40 subunits, the proteasome is a protein complex of 2.5 MDa which consists of two main components: the 20S core particle (CP) and the 19S regulatory particle (RP) (Coux *et al.*, 1996).

Proteasome configurations centered on the CP can have either one or two RPs but also one or two alternative proteasome activating complexes giving rise to a variety of proteasome complex configurations. Proteasome holo-enzymes engaged in the degradation of poly-ubiquitylated proteins require the RP, thus occur either as RP-CP or RP-CP-RP, also termed the 26S and the 30S proteasome, respectively (Eytan *et al.*, 1989).

Structure of the 20S Core Particle

The proteasome belongs to the family of threonine proteases and its maturation follows the concept of zymogen activation upon which proteases are activated, once they arrive at their destination. With a molecular mass of 700 kDa, the CP is composed of seven distinct α and β subunits, each of which form heptameric rings stacked into a barrel composed of two outer α rings and two inner β rings (Groll *et al.*, 1997). The maturation of the CP involves the dimerization of two inactive precursor complexes, resembling two half-CPs. Half-CPs consist of an α ring and β ring with five of the seven β subunits synthesized with propeptides. With the dimerization of two half-CPs into the pre-holo CP, the autocatalytic processing of the propeptides is triggered and three β subunits contribute an active site threonine with different peptide cleavage specificities (Li *et al.*, 2007; Ramos *et al.*, 1998). CP-dedicated chaperones, namely Pac/Pba/Poc 1-4 and Ump1, assist in CP assembly. Ump1 is a natively-disordered protein, which is buried inside the pre-holo CP and later on becoming the first substrate of the nascent CP (Kusmierczyk *et al.*, 2008; Ramos & Dohmen, 2008). The α rings are the key players in CP gating. Normally CP α rings are closed, unless they are opened by the RP to allow access of protein substrates into the proteolytic cavity (Groll *et al.*, 2000).

Structure of the 19S Regulatory Particle

As “gate keeper” of the CP, the RP is the best understood proteasome activator (Rechsteiner & Hill, 2005). The RP is divided into two parts, the base and the lid subcomplexes. The RP base is composed of six ATPases of the triple A family (ATPases Associated with diverse cellular Activities), named Rpt1-6, and five non-ATPases, Rpn1, Rpn2, Rpn10, Rpn13 and Ubp6. The base Rpn subunits are involved in the recognition of the poly-ubiquitin chain and the Rpt ATPase subunits guide the unfolding and translocation of the polypeptide substrate into the CP (Finley *et al.*, 1998). In contrast to the RP base subunits, the subunits comprising the RP lid are only of the non-ATPase class: Rpn3, Rpn5-9, Rpn11 and Rpn12 (Glickman *et al.*, 1998). The main known function of the RP lid is the processing of poly-ubiquitin chains. Rpn11 contributes isopeptidase activity to recycle ubiquitin moieties from the protein substrates. Ubp6 also has ubiquitin hydrolase activity and assists in trimming poly-ubiquitin chains (Crosas *et al.*, 2006; Hanna *et al.*, 2006; Lam *et al.*, 1997; Verma *et al.*, 2002). In principle, the RP ensures that only targeted substrates are degraded by the proteasome, thereby conferring the ubiquitin- and ATP-dependence towards proteasomal protein degradation.

Two competing models exist for RP assembly (Funakoshi *et al.*, 2009; Le Tallec *et al.*, 2009; Park *et al.*, 2009; Roelofs *et al.*, 2009). The first posits that RP assembly occurs in modules independent of the CP with the help of four RP-dedicated chaperones, named Hsm3, Nas2, Nas6 and Rpn14 (Funakoshi *et al.*, 2009). In

contrast, the second model proposes that the CP serves as a scaffold for the heterohexameric ATPase ring of the RP base (Park *et al.*, 2009). The second model, however, appears less likely with regard to X-ray structure analysis showing that the RP-dedicated chaperones hinder the association between the RP base and CP α ring (Barrault *et al.*, 2012). The CP-independent assembly model is also supported by the finding that the assembly of RP base and lid can be reconstituted from recombinant proteins with the assistance of RP-dedicated chaperones but without the CP template (Beckwith *et al.*, 2013). However, the CP could serve as a platform for RP base assembly, if RP-dedicated chaperones are limiting.

Localization of the proteasome

At this point, it is important to acknowledge the importance of GFP labelling and the ease with which it has allowed localization studies to be conducted (Enenkel, 2014; Groothuis & Reits, 2005). In our species of interest, *Saccharomyces cerevisiae*, which is an excellent model organism for eukaryotic cells, GFP labelling of proteasomes is achieved by homologous recombination techniques into the chromosomal locus to convert an endogenous proteasomal subunit to a GFP-tagged version (Enenkel *et al.*, 1999; Laporte *et al.*, 2008; McDonald & Byers, 1997). Nearly all proteasomal genes are essential and could be modified by GFP fusions without interfering with their function; we prefer the CP subunits α 4 and β 5, the CP-dedicated chaperone Ump1, and the RP subunits Rpn1, Rpt1 and Rpn11 as GFP-labelled reporters, because their GFP fusion proteins are fully incorporated into the proteasomal subcomplexes. So far, ~30 subunits of the yeast proteasome were labelled with GFP. All of them reveal the same subcellular localization as thoroughly investigated by direct fluorescence microscopy in living yeast (Laporte *et al.*, 2008).

Seminal studies on proteasome localization in vertebrate cells were performed by Werner Franke's and Wolfgang Baumeister's laboratories in the early 1990s. Proteasomes were mainly detected in the nuclei of *Xenopus laevis* oocytes and cultured mammalian cells (Amsterdam *et al.*, 1993; Hugle *et al.*, 1983; Kleinschmidt *et al.*, 1983). Later investigations reported a shift towards cytoplasmic proteasomes dependent on the type of the cell line and the density of the cells (Wojcik & DeMartino, 2003). Proteasome localization also varies with the growth phase in yeast (Laporte *et al.*, 2008; Weberruss *et al.*, 2013). In growing yeast at logarithmic phase (OD~1), proteasomes are primarily nuclear. During the transition from proliferation to quiescence and the entrance into stationary phase (OD>3), proteasomes deplete from the nucleus and accumulate at the NE/ER in membraneless organelles. These enigmatic structures of proteasomes were initially observed by Isabelle Sagot and her co-workers, who coined the term proteasome storage granules (PSGs) (Laporte *et al.*, 2008). With prolonged quiescence, one to two PSGs with a diameter of ~0.2 to 0.5 μ m pinch off the NE into the cytoplasm. The PSGs are motile and stable in yeast cultures and are kept in quiescence for several weeks. If quiescent yeast cells are allowed to resume growth by replacing the glucose-depleted medium with glucose-rich medium, the PSG rapidly clears and the proteasomes are relocated into the nucleus within a few minutes.

Studies with mammalian cancer cell lines also exploited GFP-labelling techniques and fluorescence recovery after photobleaching

experiments. The experiments suggested that nuclear transport of GFP-labelled CP across the NE was inefficient. Only the mitotic breakdown of the NE and its reassembly after mitosis allowed nuclear uptake of proteasomes (Reits *et al.*, 1997). However, this nuclear uptake mechanism cannot explain the predominant nuclear localization of proteasomes in yeast cells which divide without mitotic breakdown of the NE. Proteasomes are the second most abundant protein complexes in eukaryotic cells and require continuous synthesis within the cytoplasm and nuclear import during cell division (Weberruss *et al.*, 2013). The most common route for protein complexes to cross the NE in an organism with closed mitosis is through nuclear pore complexes (NPC). Before we address this pathway for yeast proteasomes, we will shortly summarize the concept of nuclear transport through the NPC, a pathway conserved from yeast to human.

Nuclear import in proliferating yeast cells

The NE is embellished with NPCs which regulate the entry of molecules into and out of the nucleus. Their principal function is to allow free diffusion of small molecules, such as water/ions/peptides, and to block non-specific translocation of macromolecules that exceed 40kDa or a diameter larger than 5nm (Aitchison & Rout, 2012; Wentz & Rout, 2010). Translocation of larger macromolecules requires specific interactions with the NPC. Protein cargoes therefore associate with soluble transport factors, called karyopherins/importins/exportins, that themselves interact with phenylalanine-glycine rich nucleoporins (FG-Nups) decorating the NPC (Wozniak *et al.*, 1998). Importins and exportins identify their protein cargoes by nuclear localization sequences (NLSs) and nuclear export signals (NESs), which ensure their nuclear import and export, respectively. In the literature, there are variations of nuclear import and export signals, only some of which comply with the classical import/export concept. The classical concept applies for nuclear import of proteasomes. Thus, we will focus on the key components required for the classical pathway (Gorlich & Kutay, 1999).

The classical nuclear import cycle starts with the association of the importin/karyopherin $\alpha\beta$ heterodimer, called Srp1/Kap95 in yeast, with the cargo NLS. Two types of classical NLSs exist: the monopartite NLS which contains five basic amino acid residues and the bipartite NLS in which two clusters of basic residues are spaced by 10–12 indifferent residues. Importin α has the NLS-binding grooves, and importin β mediates the interaction with FG-Nups. The directionality of nuclear transport is dictated by the Ran-GTP/GDP gradient across the NE. Ran is a small GTPase, named Gsp1 in yeast. Ran exists in its GTP-bound state in the nucleus and in its GDP-bound state in the cytoplasm due to the actions of the Ran guanine nucleotide exchange factor (RanGEF) and the RanGTPase activating protein (RanGAP) in the nucleoplasm and cytoplasm, respectively (Gorlich & Kutay, 1999; Moore & Blobel, 1993). In the nucleus, the cargo-importin $\alpha\beta$ complex encounters RanGTP, which results in the release of the cargo (Rexach & Blobel, 1995). Cargo-free importin $\alpha\beta$ is recycled into the cytoplasm for the next round of nuclear import.

Nuclear import of proteasomes during proliferation

Our studies in yeast strongly suggest that newly synthesized proteasomes are imported from the cytosol into the nucleus as inactive precursor complexes and that the maturation of nuclear CP proceeds to completion post-import (Lehmann *et al.*, 2002). Although electron microscopy studies have shown that the NPC could expand to accommodate the longitudinal passage of the 30S proteasome, the permeability barriers towards macromolecules such as CP precursor complexes and RP assembly modules must be overcome by specific importins/karyopherins (Pante & Kann, 2002). Several classical NLSs exist within the N-termini of distinct α subunits which were proposed to be either accessible rendering the CP in an import-competent conformation, or to be masked rendering the CP in an import-incompatible conformation (Tanaka *et al.*, 1990). Indeed, recent cryo-EM structure analysis revealed flexible and less structured α ring surfaces in Ump1-associated CP precursor complexes (Kock *et al.*, 2015), in compliance with our finding that importin α recognizes CP precursor complexes but not mature CP with closed α rings (Lehmann *et al.*, 2002). Our model upon which CP precursor complexes are imported into the nucleus was supported by the following observations (Figure 1). First, when tagged with GFP, Ump1 is predominantly nuclear in spite of the fact that CP precursor complexes are assembled from nascent subunits in the cytoplasm. Second, in importin α mutants namely *srp1-49* but not in *srp1-31*, several groups found that the CP is mislocalized to the cytoplasm, providing another piece of evidence for the classical import pathway of proteasomes. Unprocessed and incompletely processed $\beta 5$ subunits, crucial determinants of CP precursor complexes and pre-holo-CP, respectively, accumulate in *srp1-49* mutants, while precursors of $\beta 5$ subunits are hardly detectable in wild type cells (Lehmann *et al.*, 2002). Third, when CP maturation is delayed by *UMP1* deletion, all CP reporter proteins accumulate in the nucleus, although half of the CP is not fully matured and most likely exists as pre-holo-CP (Fehlker *et al.*, 2003; Lehmann *et al.*, 2008).

In the case of the RP, functional NLSs were identified in RP base subunits Rpn2 and Rpt2 and are recognized by importin α (Figure 2). The deletion of the Rpn2 NLS caused a temperature sensitive phenotype and mislocalizations of the RP base into cytosolic foci, whereas the deletion of the Rpt2 NLS was compensated by the presence of the Rpn2 NLS. At permissive temperatures, neither the Rpn2 nor the Rpt2 NLS deletion had severe impact on nuclear proteasome localization suggesting a redundancy of proteasomal NLSs (Wendler *et al.*, 2004). Isono *et al.* (2007) later confirmed that Rpn2 provides a crucial NLS to aid nuclear import of the RP base and that the lid is separately imported. The nuclear import of the RP lid also requires importin α , though no classical NLS has been identified within RP lid subunits; rather Sts1, a short-lived protein that itself contains a classical NLS, associates with Rpn11 to facilitate nuclear import of the RP lid by importin $\alpha\beta$ (Chen *et al.*, 2011). In accordance, deletion of the Sts1 NLS has downstream effects on the nuclear localization of RP lid in addition to RP base and CP, which suggests that proteasomes could also be transported as holoenzymes (Chen & Madura, 2014b). In order to ensure comparable

Figure 1. Nuclear import of CP precursor complexes by the classical importin/karyopherin $\alpha\beta$ pathway in proliferating yeast. The α rings with the classical NLS are depicted in red. The β rings with propeptides are depicted in blue. The CP-dedicated chaperone and maturation factor Ump1 is depicted in yellow. The completion of CP maturation occurs in the nucleus with the degradation of Ump1.

Figure 2. Nuclear import of RP base and lid subcomplexes by the classical importin/karyopherin $\alpha\beta$ pathway. Rpn2/Rpt2 and Sts1 confer classical NLS to the RP base and lid complex, respectively. Sts1 is short-lived and most likely degraded with RP-CP assembly.

stoichiometry of proteasomal subcomplexes in the nucleus and similar kinetics by which they are imported into the nucleus, it is reasonable that importin $\alpha\beta$ is used as common nuclear import receptor.

Recent fluorescence correlation spectroscopy studies also support the conclusion that proteasomes can be imported into the nucleus as holo-enzymes (Pack *et al.*, 2014; Figure 3). However, the maturation state of the GFP-labelled proteasomes was unclear. Possibly, pre-holo-CP are the real nuclear transport intermediates which degrade Ump1 and Sts1 upon the arrival in the nucleus with the completion of proteasome maturation.

The enigma of proteasome storage granules

When cells experience nutrient exhaustion or enter quiescence, a drastic change in proteasome localization is observed. In prolonged quiescence, proteasomes deplete from the nucleus and reside in motile and reversible PSGs in the cytoplasm (Laporte *et al.*, 2008). Upon addition of glucose, cells receive the signal to resume proliferation, and PSGs dissolve rapidly, where proteasomes are relocated in the nucleus. How PSGs are organized is not understood. Premature PSG formation in proliferating cells was found to depend on vacuolar ATPases and linked premature PSG formation with dysregulation of the intracellular pH. In view of that, PSGs could serve as storage depots for mature proteasomes in quiescence, to protect the proteasome from cellular stress and to be eliminated by autophagocytosis (Peters *et al.*, 2013). The storage of proteasomes during quiescence would also alleviate energy-consuming synthesis of new proteasomes with cell proliferation (Laporte *et al.*, 2008).

The formation of PSG-like structures is also observed by chemical inhibition of proteasomes in mammalian cells or temperature sensitive proteasome mutants in yeast, conditions which result in cell cycle arrest. In spite of the differences between chemically-induced cell cycle arrest and quiescence, inhibited proteasomes are

sequestered into juxta nuclear quality control compartments (JUNQs), situated at the cytoplasmic side of the NE and behaving similar to PSGs (Kaganovich *et al.*, 2008; Weberruss *et al.*, 2013). When the cell cycle-arrested mutants were allowed to resume growth at permissive temperatures or upon withdrawal of proteasome inhibition, JUNQs were seen to dissolve like the PSG. In the context of these studies in cell-cycle arrested yeast and mammalian cells, poly-ubiquitinated proteins were found to be accumulated in the JUNQ as well, suggesting that the JUNQ provides a major place for proteolysis (Kaganovich *et al.*, 2008). All studies on the JUNQ and PSGs agree that these enigmatic organelles serve protective functions. Their presence protects cells against proteo- and genotoxic stress and confers cell fitness during aging. Post-translational modifications such as N-acetylation also play a role in PSG organization, but their targets are unknown (Saunier *et al.*, 2013; van Deventer *et al.*, 2015; Weberruss *et al.*, 2013).

Nuclear import of proteasomes upon exit from quiescence

Though the CP and RP co-localize in the PSG, they seem to be loosely associated. Conflicting reports exist about the stability of RP-CP assemblies in lysates of quiescent cells (Bajorek *et al.*, 2003; Hanna *et al.*, 2012; Weberruss *et al.*, 2013). The finding that RP-CP assemblies are less stable coincides with the decline in ATP during quiescence as well as the reduced proclivity of the proteasome to degrade poly-ubiquitinated substrate. Instead of an association of the CP with the RP, most CP is seen interacting with Blm10, a conserved 240 kDa HEAT repeat protein (Weberruss *et al.*, 2013). Upon exit from quiescence, the PSGs rapidly clear and mature proteasomes are imported into the nucleus within a few minutes. The imported proteasomes must be matured and assembled, as time does not permit the new synthesis of precursor complexes (Laporte *et al.*, 2008). Here, Blm10 plays an important role and represents the first characterized nuclear transporter which particularly facilitates nuclear import of mature CP (Figure 4). Quiescent *blm10Δ*

Figure 3. Assembled holo-proteasomes with RP-CP-RP configuration pass the nuclear pore.

Figure 4. Blm10-dependent nuclear import of mature CP. In quiescent yeast cells mature CP is stored in reversible and motile granules in the cytoplasm, which rapidly clear with the resumption of growth. Blm10 mediates the nuclear import of mature CP.

mutants exhibit a significant delay in resuming cell growth due to the deficit in mature CP in the nucleus. Furthermore, Blm10 binds FG-Nups and GTP-bound Ran and dissociates from the CP upon interaction with RanGTP, suggesting that Blm10 shares functional similarities with Kap95, the classical importin β (Weberruss *et al.*, 2013). Along this line, Blm10 belongs to the HEAT repeat family with α -solenoid fold, a structural feature shared by β karyopherins/importins (Huber & Groll, 2012). During cell proliferation, Blm10 is also expressed but to a much lesser extent (Weberruss *et al.*, 2013). Only a minor fraction of the CP, pre-holo-CP and CP precursor complexes is associated with Blm10 in growing yeast. The Blm10-bound fraction significantly increases under geno- and proteotoxic stress suggesting a high demand for nuclear proteasomes under these growth conditions (Doherty *et al.*, 2012; Fehlker *et al.*, 2003; Lehmann *et al.*, 2008). Since Blm10 associates with constitutively open or disordered CP α rings, Blm10 also plays a role in regulating α -ring gating during CP maturation (Lehmann *et al.*, 2008). The wider α ring conformation of CP-precursor complexes seems to be preferentially bound to Blm10 and importin $\alpha\beta$ by representing import intermediates. Thus, the Blm10-dependent import pathway complements the canonical nuclear import pathway.

For the RP, the import pathway upon exit from quiescence is yet to be solidified. A possible candidate for a RP-dedicated nuclear import receptor is Rpn2 which exhibits a similar α -solenoid fold as Blm10 and importin β , all of which belong to the family of HEAT-repeat proteins (Huber & Groll, 2012; Kajava, 2002).

Conclusions

In this review, we discussed the recent literature on the dynamics of the ubiquitin-proteasome system with a major focus on the proteasome. During cell proliferation a high traffic volume of proteasomes and proteasomal substrates arises between the cyto- and

nucleoplasm. In cell-cycle arrested and quiescent cells, proteasomes exit the nucleus and accumulate with poly-ubiquitylated proteins in motile and reversible PSGs in the nuclear periphery. While the basic concepts of nuclear import of proteasomes during cell proliferation and upon exit from quiescence are understood, little is known about the nuclear export of proteasomes during the transition from proliferation to quiescence. We may wonder why proteasomes exit the nucleus during quiescence. Which kind of substrates will be available in the cytoplasm, once proteasomes are sequestered into the PSG? Possibly, PSG-resident proteasomes are starving for newly synthesized proteins which arise with the resumption of cell proliferation.

The dynamics of proteasomes and their substrates are fascinating and will inspire our discussions and experiments in the future.

Author contributions

M.C. prepared the first draft of the manuscript and agreed to the final content.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by grants from NSERC (4422666-2011) awarded to M.C. and C.E. and from CIHR (325477) awarded to C.E.

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgement

We thank Zhu Chao (Jerry) Gu and Julianne Burcoglu for critical reading of the manuscript.

References

- Aitchison JD, Rout MP: **The yeast nuclear pore complex and transport through it.** *Genetics*. 2012; **190**(3): 855–883.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Amsterdam A, Pitzer F, Baumeister W: **Changes in intracellular localization of proteasomes in immortalized ovarian granulosa cells during mitosis associated with a role in cell cycle control.** *Proc Natl Acad Sci U S A*. 1993; **90**(1): 99–103.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bajorek M, Finley D, Glickman MH: **Proteasome disassembly and downregulation is correlated with viability during stationary phase.** *Curr Biol*. 2003; **13**(13): 1140–1144.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Barraut MB, Richet N, Godard C, *et al.*: **Dual functions of the Hsm3 protein in chaperoning and scaffolding regulatory particle subunits during the proteasome assembly.** *Proc Natl Acad Sci U S A*. 2012; **109**(17): E1001–1010.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Beckwith R, Estrin E, Worden EJ, *et al.*: **Reconstitution of the 26S proteasome reveals functional asymmetries in its AAA+ unfoldase.** *Nat Struct Mol Biol*. 2013; **20**(10): 1164–1172.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen L, Madura K: **Degradation of specific nuclear proteins occurs in the cytoplasm in *Saccharomyces cerevisiae*.** *Genetics*. 2014a; **197**(1): 193–197.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen L, Madura K: **Yeast importin- α (Srp1) performs distinct roles in the import of nuclear proteins and in targeting proteasomes to the nucleus.** *J Biol Chem*. 2014b; **289**(46): 32339–32352.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chen L, Romero L, Chuang SM, *et al.*: **Sts1 plays a key role in targeting proteasomes to the nucleus.** *J Biol Chem*. 2011; **286**(4): 3104–3118.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ciechanover A, Brundin P: **The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg.** *Neuron*. 2003; **40**(2): 427–446.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Coux O, Tanaka K, Goldberg AL: **Structure and functions of the 20S and 26S proteasomes.** *Annu Rev Biochem*. 1996; **65**: 801–847.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Crosas B, Hanna J, Kirkpatrick DS, *et al.*: **Ubiquitin chains are remodeled at the proteasome by opposing ubiquitin ligase and deubiquitinating activities.** *Cell*. 2006; **127**(7): 1401–1413.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Doherty KM, Pride LD, Lukose J, *et al.*: **Loss of a 20S proteasome activator in *Saccharomyces cerevisiae* downregulates genes important for genomic integrity, increases DNA damage, and selectively sensitizes cells to agents with diverse mechanisms of action.** *G3 (Bethesda)*. 2012; **2**(8): 943–959.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Enenkel C: **Proteasome dynamics.** *Biochim Biophys Acta*. 2014; **1843**(1): 39–46.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Enenkel C, Lehmann A, Kloetzel PM: **Subcellular distribution of proteasomes implicates a major location of protein degradation in the nuclear envelope-ER network in yeast.** *EMBO J*. 1998; **17**(21): 6144–6154.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Enenkel C, Lehmann A, Kloetzel PM: **GFP-labelling of 26S proteasomes in living yeast: insight into proteasomal functions at the nuclear envelope/rough ER.** *Mol Biol Rep*. 1999; **26**(1–2): 131–135.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Eytan E, Ganoth D, Armon T, *et al.*: **ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin.** *Proc Natl Acad Sci U S A*. 1989; **86**(20): 7751–7755.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fehlker M, Wendler P, Lehmann A, *et al.*: **Blm3 is part of nascent proteasomes and is involved in a late stage of nuclear proteasome assembly.** *EMBO Rep*. 2003; **4**(10): 959–963.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Finley D: **Recognition and processing of ubiquitin-protein conjugates by the proteasome.** *Annu Rev Biochem*. 2009; **78**: 477–513.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Finley D, Tanaka K, Mann C, *et al.*: **Unified nomenclature for subunits of the *Saccharomyces cerevisiae* proteasome regulatory particle.** *Trends Biochem Sci*. 1998; **23**(7): 244–245.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Finley D, Ulrich HD, Sommer T, *et al.*: **The ubiquitin-proteasome system of *Saccharomyces cerevisiae*.** *Genetics*. 2012; **192**(2): 319–360.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fishbain S, Inobe T, Israeli E, *et al.*: **Sequence composition of disordered regions fine-tunes protein half-life.** *Nat Struct Mol Biol*. 2015; **22**(3): 214–221.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Funakoshi M, Tomko RJ Jr, Kobayashi H, *et al.*: **Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base.** *Cell*. 2009; **137**(5): 887–899.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Glickman MH, Rubin DM, Fried VA, *et al.*: **The regulatory particle of the *Saccharomyces cerevisiae* proteasome.** *Mol Cell Biol*. 1998; **18**(6): 3149–3162.
[PubMed Abstract](#) | [Free Full Text](#)
- Goldberg AL: **Protein degradation and protection against misfolded or damaged proteins.** *Nature*. 2003; **426**(6968): 895–899.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gorlich D, Kutay U: **Transport between the cell nucleus and the cytoplasm.** *Annu Rev Cell Dev Biol*. 1999; **15**: 607–660.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Groll M, Bajorek M, Köhler A, *et al.*: **A gated channel into the proteasome core particle.** *Nat Struct Biol*. 2000; **7**(11): 1062–1067.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Groll M, Ditzel L, Löwe J, *et al.*: **Structure of 20S proteasome from yeast at 2.4 Å resolution.** *Nature*. 1997; **386**(6624): 463–471.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Groothuis TA, Reits EA: **Monitoring the distribution and dynamics of proteasomes in living cells.** *Methods Enzymol*. 2005; **399**: 549–563.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanna J, Hathaway NA, Tone Y, *et al.*: **Deubiquitinating enzyme Ubp6 functions noncatalytically to delay proteasomal degradation.** *Cell*. 2006; **127**(1): 99–111.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hanna J, Waterman D, Boselli M, *et al.*: **Spg5 protein regulates the proteasome in quiescence.** *J Biol Chem*. 2012; **287**(41): 34400–34409.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Harper JW, Schulman BA: **Structural complexity in ubiquitin recognition.** *Cell*. 2006; **124**(6): 1133–1136.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hershko A, Ciechanover A: **The ubiquitin system.** *Annu Rev Biochem*. 1998; **67**: 425–479.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huber EM, Groll M: **The 19S cap puzzle: a new jigsaw piece.** *Structure*. 2012; **20**(3): 387–388.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hügler B, Kleinschmidt JA, Franke WW: **The 22 S cylinder particles of *Xenopus laevis*. II. Immunological characterization and localization of their proteins in tissues and cultured cells.** *Eur J Cell Biol*. 1983; **32**(1): 157–163.
[PubMed Abstract](#)
- Huh WK, Falvo JV, Gerke LC, *et al.*: **Global analysis of protein localization in budding yeast.** *Nature*. 2003; **425**(6959): 686–691.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Isono E, Nishihara K, Saeki Y, *et al.*: **The assembly pathway of the 19S regulatory particle of the yeast 26S proteasome.** *Mol Biol Cell*. 2007; **18**(2): 569–580.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kaganovich D, Kopito R, Frydman J: **Misfolded proteins partition between two distinct quality control compartments.** *Nature*. 2008; **454**(7208): 1088–1095.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kajava AV: **What curves alpha-solenoids? Evidence for an alpha-helical toroid structure of Rpn1 and Rpn2 proteins of the 26 S proteasome.** *J Biol Chem*. 2002; **277**(51): 49791–49798.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kirschner M: **Intracellular proteolysis.** *Trends Cell Biol*. 1999; **9**(12): M42–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kleinschmidt JA, Hügler B, Grund C, *et al.*: **The 22 S cylinder particles of *Xenopus laevis*. I. Biochemical and electron microscopic characterization.** *Eur J Cell Biol*. 1983; **32**(1): 143–156.
[PubMed Abstract](#)
- Knecht E, Rivett J: **Intracellular Localization of Proteasomes.** In *Proteasomes: The World of Regulatory Proteolysis*. W Hilt, and DH Wolf, eds. (Georgetown, Texas, USA: Landes Bioscience, Eurekah.com). 2000; 176–185.
[Reference Source](#)
- Kock M, Nunes MM, Hemann M, *et al.*: **Proteasome assembly from 15S precursors involves major conformational changes and recycling of the Pba1-Pba2 chaperone.** *Nat Commun*. 2015; **6**: 6123.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kusmierczyk AR, Kunjappu MJ, Funakoshi M, *et al.*: **A multimeric assembly factor controls the formation of alternative 20S proteasomes.** *Nat Struct Mol Biol*. 2008; **15**(3): 237–244.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lam YA, Xu W, DeMartino GN, *et al.*: **Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome.** *Nature*. 1997; **385**(6618): 737–740.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Laporte D, Salin B, Daignan-Fornier B, *et al.*: **Reversible cytoplasmic localization of the proteasome in quiescent yeast cells.** *J Cell Biol*. 2008; **181**(5): 737–745.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Le Tallec B, Barraut MB, Guérois R, *et al.*: **Hsm3/S5b participates in the assembly pathway of the 19S regulatory particle of the proteasome.** *Mol Cell*.

- 2009; **33**(3): 389–399.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lehmann A, Janek K, Braun B, *et al.*: **20 S proteasomes are imported as precursor complexes into the nucleus of yeast.** *J Mol Biol.* 2002; **317**(3): 401–413.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lehmann A, Jechow K, Enenkel C: **Blm10 binds to pre-activated proteasome core particles with open gate conformation.** *EMBO Rep.* 2008; **9**(12): 1237–1243.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Li X, Kusmierczyk AR, Wong P, *et al.*: **beta-Subunit appendages promote 20S proteasome assembly by overcoming an Ump1-dependent checkpoint.** *EMBO J.* 2007; **26**(9): 2339–2349.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McDonald HB, Byers B: **A proteasome cap subunit required for spindle pole body duplication in yeast.** *J Cell Biol.* 1997; **137**(3): 539–553.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Moore MS, Blobel G: **The GTP-binding protein Ran/TC4 is required for protein import into the nucleus.** *Nature.* 1993; **365**(6447): 661–663.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Pack CG, Yukil H, Toh-e A, *et al.*: **Quantitative live-cell imaging reveals spatio-temporal dynamics and cytoplasmic assembly of the 26S proteasome.** *Nat Commun.* 2014; **5**: 3396.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Panté N, Kann M: **Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm.** *Mol Biol Cell.* 2002; **13**(2): 425–434.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Park S, Roelofs J, Kim W, *et al.*: **Hexameric assembly of the proteasomal ATPases is templated through their C termini.** *Nature.* 2009; **459**(7248): 866–870.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Park SH, Kukushkin Y, Gupta R, *et al.*: **PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone.** *Cell.* 2013; **154**(1): 134–145.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Peters LZ, Hazan R, Breker M, *et al.*: **Formation and dissociation of proteasome storage granules are regulated by cytosolic pH.** *J Cell Biol.* 2013; **201**(5): 663–671.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Prasad R, Kawaguchi S, Ng DT: **A nucleus-based quality control mechanism for cytosolic proteins.** *Mol Biol Cell.* 2010; **21**(13): 2117–2127.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ramos PC, Dohmen RJ: **PACemakers of proteasome core particle assembly.** *Structure.* 2008; **16**(9): 1296–1304.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ramos PC, Hockendorff J, Johnson ES, *et al.*: **Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly.** *Cell.* 1998; **92**(4): 489–499.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rechsteiner M, Hill CP: **Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors.** *Trends Cell Biol.* 2005; **15**(1): 27–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Reits EA, Benham AM, Plougastel B, *et al.*: **Dynamics of proteasome distribution in living cells.** *EMBO J.* 1997; **16**(20): 6087–6094.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rendueles PS, Wolf DH: **Proteinase function in yeast: biochemical and genetic approaches to a central mechanism of post-translational control in the eukaryote cell.** *FEMS Microbiol Rev.* 1988; **4**(1): 17–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rexach M, Blobel G: **Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins.** *Cell.* 1995; **83**(5): 683–692.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Roelofs J, Park S, Haas W, *et al.*: **Chaperone-mediated pathway of proteasome regulatory particle assembly.** *Nature.* 2009; **459**(7248): 861–865.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Russell SJ, Steger KA, Johnston SA: **Subcellular localization, stoichiometry, and protein levels of 26 S proteasome subunits in yeast.** *J Biol Chem.* 1999; **274**(31): 21943–21952.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Sahtoe DD, Sixma TK: **Layers of DUB regulation.** *Trends Biochem Sci.* 2015; **40**(8): 456–467.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Salomons FA, Acs K, Dantuma NP: **Illuminating the ubiquitin/proteasome system.** *Exp Cell Res.* 2010; **316**(8): 1289–1295.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Saunier R, Esposito M, Dassa EP, *et al.*: **Integrity of the *Saccharomyces cerevisiae* Rpn11 protein is critical for formation of proteasome storage granules (PSG) and survival in stationary phase.** *PLoS One.* 2013; **8**(8): e70357.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sugaya K, Ishihara Y, Inoue S: **Nuclear localization of ubiquitin-activating enzyme Uba1 is characterized in its mammalian temperature-sensitive mutant.** *Genes Cells.* 2015.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Sugaya K, Ishihara Y, Inoue S, *et al.*: **Characterization of ubiquitin-activating enzyme Uba1 in the nucleus by its mammalian temperature-sensitive mutant.** *PLoS One.* 2014; **9**(5): e96666.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tanaka K, Yoshimura T, Tamura T, *et al.*: **Possible mechanism of nuclear translocation of proteasomes.** *FEBS Lett.* 1990; **271**(1–2): 41–46.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tsien RY: **The green fluorescent protein.** *Annu Rev Biochem.* 1998; **67**: 509–544.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vabulas RM, Hartl FU: **Protein synthesis upon acute nutrient restriction relies on proteasome function.** *Science.* 2005; **310**(5756): 1960–1963.
[PubMed Abstract](#) | [Publisher Full Text](#)
- van Deventer S, Menendez-Benito V, van Leeuwen F, *et al.*: **N-terminal acetylation and replicative age affect proteasome localization and cell fitness during aging.** *J Cell Sci.* 2015; **128**(1): 109–117.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Verma R, Aravind L, Oania R, *et al.*: **Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome.** *Science.* 2002; **298**(5593): 611–615.
[PubMed Abstract](#) | [Publisher Full Text](#)
- von Mikecz A: **The nuclear ubiquitin-proteasome system.** *J Cell Sci.* 2006; **119**(Pt 10): 1977–1984.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Weberruss MH, Savulescu AF, Jando J, *et al.*: **Blm10 facilitates nuclear import of proteasome core particles.** *EMBO J.* 2013; **32**(20): 2697–2707.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wendler P, Lehmann A, Janek K, *et al.*: **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.* 2004; **279**(36): 37751–37762.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wente SR, Rout MP: **The nuclear pore complex and nuclear transport.** *Cold Spring Harb Perspect Biol.* 2010; **2**(10): a000562.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wojcik C, DeMartino GN: **Intracellular localization of proteasomes.** *Int J Biochem Cell Biol.* 2003; **35**(5): 579–589.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wozniak RW, Rout MP, Aitchison JD: **Karyopherins and kissing cousins.** *Trends Cell Biol.* 1998; **8**(5): 184–188.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 20 August 2015

<https://doi.org/10.5256/f1000research.7349.r9634>

© 2015 Ramos P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Paula C. Ramos

Institute for Genetics, University of Cologne, Cologne, Germany

The dynamics of proteasome composition and subcellular distribution is an important subject with far-reaching implication. Recently, substantial progress has been made on this subject, while many open questions remain. It is therefore of considerable value that Chowdhury and Enenkel have summarized and commented the current literature on this subject in this nice and timely review. I have only a few comments that should be easy to address:

1. In the second paragraph of the introduction it is stated that long-lived proteins are degraded in the lysosome, whereas short-lived proteins are degraded by the proteasome. While experimental data suggest that this is true for the bulk of the proteins in *S. cerevisiae* (e.g. [Lee and Goldberg, 1996](#)), there is evidence from inhibitor studies with mammalian cells indicating that also long-lived proteins are among the proteasome substrates ([Rock et al. 1994](#)). Thus, while the statement in the introduction goes in the right direction, it would be better to soften it a bit.
2. The sentence mentioning that Ump1 is a natively-disordered protein refers to papers from 2008. Reports that provided the first data supporting this notion, however, were published in 2013 by two independent groups (see below). Therefore, I would suggest to refer to these references in this context

Ref.1: Sá-Moura B, S.A., Fraga J, Fernandes H, Abreu IA, Botelho HM, Gomes CH, Marques AJ, Dohmen RJ, Ramos PC, Macedo-Ribeiro S. [Biochemical and Biophysical Characterization of Recombinant Yeast Proteasome Maturation Factor Ump1](#). Computational and Structural Biotechnology Journal 7, e201304006 (2013)

Ref 2: Uekusa, Y. et al. [Backbone ¹H, ¹³C and ¹⁵N assignments of yeast Ump1, an intrinsically disordered protein that functions as a proteasome assembly chaperone](#). Biomol NMR Assign (2013).

3. The structural data on 15S precursor complex were not obtained via cryo-EM but rather by negative stain EM (reference Kock et al. 2015).

4. When the authors discuss that the alpha ring of the 15S precursor complex is more relaxed and that would support the idea that the NLS would be more available for transportation from the cytoplasm into the nucleus, the authors have not considered or discussed the location of the chaperones Pba1-Pba2, that covers the surface corresponding to the pore of the 20S proteasome and thus would hide the NLS in this precursor form. I suggest to consider this point in the respective discussion and possibly to add Pba1-Pba2 to the 15S precursor cartoon shown in figure 1.
5. It is mentioned (in the paragraph on “Nuclear import of proteasomes during proliferation”) that several groups found mislocalization of the CP to the cytoplasm, but no references are provided to support this notion.
6. It does not become entirely clear whether the authors propose that PSG and JUNQ are different names for one and the same compartment. Some of the remarks seem to suggest that, but this question is not explicitly raised and addressed. Maybe the authors could clarify this issue and their view on it a bit more.
7. While the authors describe PSGs as follows: “yeast proteasomes gather in proteasome storage granules (PSGs) at the nuclear envelope (NE) and endoplasmic reticulum (ER) membrane”, from which “with prolonged quiescence... pinch off the NE into the cytoplasm”, in the study that originally coined the term PSG (Laporte et al. 2008), they were defined as “motile cytoplasmic structures” that are “not associated with specific organelles or any detectable membrane”. In fact, these PSGs were distinguished from locations of proteasomes at or close to the nuclear periphery (e.g. Figure 2B of that study). It thus appears as if Chowdhury and Enenkel use a different or wider definition of PSGs than the one used originally by Laporte et al., which is relevant to the previous issue (6.). If so, it would be helpful to propose and explain the reasons for such an expansion of the definition of PSG.
8. It is probably not ideal to describe PSGs as organelles.
9. While the entire process of ubiquitylation is ATP-dependent, of the three types of enzymes involved, only E1 is an ATP-dependent enzyme, E2 and E3 are not.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Sep 2015

Cordula Enenkel, University of Toronto, Toronto, Canada

Thanks for the helpful comments which were all considered in the revised version of our review.

With regard to point 7, Laporte *et al.* (2008) report the depletion of nuclear proteasomes

during the transition from logarithmic to stationary phase and their accumulation at the NE / ER in dots close to the nuclear periphery. After 4-5 days in stationary phase the dots are mainly found in the cytoplasm and coined PSG (Figure 1 of that work).

I also clarified that PSG and JUNQ may be one and the same structure as shown by supplementary data in our work (Weberruss *et al.* 2013).

Competing Interests: No competing interests were disclosed.

Reviewer Report 14 August 2015

<https://doi.org/10.5256/f1000research.7349.r9988>

© 2015 Coffino P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Philip Coffino

Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA, USA

Scope of this review and its intended readership

The proteasome is a big and complex protein machine. This review centers on questions of its subcellular localization and where various steps in its assembly process happen. The basic question is inescapable and interesting: Proteins are made in the cytoplasm, but many or most proteasomes are found in the nucleus. How do large proteasome assemblies or subassemblies cross the nuclear membrane and where do they get put together (or sometimes taken apart)?

Presenting these matters requires background in two areas: 1. The structure and assembly of proteasomes and 2. Nuclear pore trafficking. Both are well-explored subjects, and some background on these matters needs to be presented. However, most readers of this review will bring some knowledge of these subjects, and wish to learn here about the specifics of the core subject, proteasome intracellular trafficking. I and perhaps other readers would prefer more on this and less (if space limitations require) of general background information. Additionally, how about comparing proteasome nuclear traffic and that of ribosomes? Ribosomes and proteasomes are the big machines found ubiquitously in eukaryotes. What common or distinct themes govern their subcellular movement and assembly?

A Technical Question

Much of the primary data supporting the conclusions described here depend on using various proteasome proteins fused to GFP in budding yeast. In interpreting these data it is critical to know whether or not such fusions alter kinetics or invoke alternate pathways compared to native controls. The paragraph headed **Localization of the proteasome** provides insufficient information related to this question.

Minor considerations

In the second paragraph of the Introduction it is said that "... long-lived proteins are degraded within the lysosome ... short-lived proteins are degraded by proteasomes ..." This seems too broad a claim- see Fuertes *et al.* (2003)[ref 1] for example- and should be modified or justified.

In the third paragraph of the Introduction: "Natively-disordered proteins also qualify as proteasome substrates and are cleaved without post-translational ubiquitin modification.", the reference which follows is about something quite different and another more relevant reference should be used.

In the first paragraph under Discussion/analysis of the literature it is said that "At least four ubiquitin molecules ... are required for a poly-ubiquitin chain to be recognized by the proteasome." No longer regarded as so categorically true, but in any case reference should be made to Thrower *et al.* (2000)² for this specific claim.

Figure 3 conveys no information and can be dropped.

Page 3, first full paragraph, right column: Change "in compliance with our finding" to "consistent with our finding".

In that same paragraph, perhaps the last sentence, "Third, when CP maturation is delayed by UMP1 deletion, all CP reporter proteins accumulate in the nucleus, although half of the CP is not fully matured and most likely exists as pre-holo-CP." could be rewritten to clarify the intended meaning and how this supports the model of nuclear import of CP precursor complexes.

Page 5, left column, second full paragraph: "...where proteasomes are relocated in the nucleus." change to : "...and proteasomes are relocated to the nucleus."

Page 5 same paragraph: "...and to be eliminated by autophagocytosis" change to "...and elimination by autophagocytosis".

References

1. Fuertes G, Villarroya A, Knecht E: Role of proteasomes in the degradation of short-lived proteins in human fibroblasts under various growth conditions. *Int J Biochem Cell Biol.* 2003; **35** (5): 651-664 [PubMed Abstract](#) | [Publisher Full Text](#) | [Reference Source](#)
2. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM: Recognition of the polyubiquitin proteolytic signal. *Embo J.* 2000; **19** (1): 94-102 [PubMed Abstract](#) | [Free Full Text](#) | [Publisher Full Text](#) | [Reference Source](#)

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Sep 2015

Cordula Enenkel, University of Toronto, Toronto, Canada

Thanks for your constructive criticism of our review which addressed the intracellular dynamics of the ubiquitin-proteasome system. We reduced some background information, though nuclear transport of proteasomes will not be intelligible without profound knowledge of proteasome structure and assembly. We also omitted detailed informations about technical approaches, which were addressed in related reviews (Burcoglu *et al.*, 2015). We answered your question with regard to direct and indirect localization studies using GFP technologies in living cells and antibodies in fixed cells. In yeast, the studies are highly consistent, while the data in mammalian cells are still difficult to reconcile.

We also appreciate your idea to compare nuclear transport and assembly of ribosomes and proteasomes. A chapter was added to address this point. However, we apologize that we can only refer to reviews about ribosome assembly and transport, because the citation of the original work would be beyond the scope of our review.

Your minor considerations were easily addressed.

Competing Interests: No competing interests were disclosed.

Reviewer Report 31 July 2015

<https://doi.org/10.5256/f1000research.7349.r9632>

© 2015 Wendler P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Petra Wendler

Gene Center Munich, Ludwig Maximilian University of Munich, Munich, Germany

This Review by Chowdhury and Enenkel nicely summarizes the current knowledge on the trafficking of proteasomes between cyto- and nucleoplasm. It's a very well written manuscript that gives a balanced and comprehensive view on localization studies on both, yeast and mammalian, proteasomes. I only have a few minor comments:

- The figures should be more informative. I would suggest to summarize all four figures in one or two figures, and to include/highlight all potential importers mentioned in the text (Kap95/Blm10/Rpn2). It would be tremendously helpful to add a figure summarizing the proteasomal localization data from yeast and mammalian cells. This figure should include which proteasomal subunits have been found where in the cell during which growth phase. It should also indicate the location of JUNQ and PSGs.
- Although the 20S chaperones Pba1/2 are mentioned, I am missing a discussion of their role in proteasomal localization. Binding of Pba1/2 blocs the access to the NLS of the alpha ring, as shown by Stadtmueller *et al.* (2012) and Kock *et al.* (2015). They thus seem to prevent

nuclear import of proteasomal precursors.

- The authors incorrectly referred to "cryo-EM structure analysis of Ump1-associated CP precursor complexes" (Kock *et al.*, 2015). This should be changed to "EM structure analysis...". Furthermore this point can be strengthened by also citing Wani *et al.* (2015).

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Sep 2015

Cordula Enenkel, University of Toronto, Toronto, Canada

Thanks for your comments which were very supportive of our review.

According to your suggestions we renewed the figures and added more information about PSG and JUNQ. We also discussed possible functions of Pac1/2 in CP assembly and nuclear import.

Although it would be very helpful to summarize proteasomal localization data from yeast and mammalian cells in a table, it is difficult to reconcile the data in the mammalian system. Proteasome localizations depend on the mammalian cell line, the growth conditions, the fixation conditions and antibodies used in these studies. We prefer to mention these inconsistencies in the text. In yeast, proteasome localizations are independent of the reporter subunit as monitored by direct and indirect fluorescence microscopy.

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research