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Review

Proteasome dynamics

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ABSTRACT

Proteasomes are highly conserved multisubunit protease complexes and occur in the cyto- and nucleoplasm of eukaryotic cells. In dividing cells proteasomes exist as holoenzymes and primarily localize in the nucleus. During quiescence they dissociate into proteolytic core and regulatory complexes and are sequestered into motile cytosolic clusters. Proteasome clusters rapidly clear upon the exit from quiescence, where proteasome core and regulatory complexes reassemble and localize to the nucleus again. The mechanisms underlying proteasome transport and assembly are not yet understood. Here, I summarize our present knowledge about nuclear transport and assembly of proteasomes in yeast and project our studies in this eukaryotic model organism to the mammalian cell system. This article is part of a Special Issue entitled: Ubiquitin-Proteasome System.

Contents

1. Introduction

Almost thirty years ago, Varshavsky and co-workers discovered that ubiquitin-dependent protein turnover is instrumental in the regulation of cell cycle progression and gene expression [1]. A few years later, yeast genetics by Dieter Wolf and his co-workers revealed that the proteasome, a highly conserved multi-subunit protease, is the key enzyme for the degradation of proteins that are covalently linked with poly-ubiquitin chains [2]. In this proteolytic system, ubiquitin serves as degradation signal and the proteasome as the degrading enzyme. It accounts for 80–90% of protein breakdown as estimated in cultured mammalian cells. Its substrates comprise a large variety of short-lived proteins that are conjugated to a poly-ubiquitin chain [3].

Proteins associated with nuclear functions, such as cyclins, cyclin-dependent kinase inhibitors, and transcription factors (NF-κB, IκB and p53), were among the first physiological proteasomal substrates to be identified [4,5]. Subsequently, cytoplasmic proteins whose turnover was also dependent on the proteasome were identified. Among these cytosolic proteins are newly synthesized proteins that do not reach their intended conformation or location [6].

Decreased proteasome activity associated with aging may account for the burden of aggregation-prone, age-dependent protein substrates, a shared hallmark of neurodegenerative diseases [7]. However, it is still unclear whether proteasome dysfunction is a cause or a consequence of these neurodegenerative diseases [8].

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In this review I will focus on our present knowledge about the transport of proteasomes between the nucleo- and cytoplasm, a process tightly linked with the assembly and disassembly of these multi-subunit protease complexes.

2. The diversity of proteasome complexes

Throughout the eukaryotic kingdom proteasome complexes exist in different configurations. All complexes have a catalytic core particle (CP), but they differ in the number of associated regulatory particles (RP) and accessory proteins. Named on the basis of their sedimentation coefficients, the 26S and 30S proteasomes contain one CP (20S) associated with one RP (19S) or two RP complexes, respectively. In addition to the standard proteasome that degrades all kinds of short-lived proteins, cytokine-inducible isoforms of the proteasome serve immune-specific tasks in mammalian cells [9].

More than thirty different subunits are present in the mature RP-CP assemblies. The CP contains seven distinct α and seven distinct β subunits. These subunits are arranged into seven-membered rings that are stacked to give a barrel-shaped particle with α7β7β7α7 configuration. The active site residues reside in the CP cavity formed by both β rings.

The α rings are responsible for gating the central channels on both sides of the CP barrel. Normally, the α ring gates are in a closed conformation [10]. Thus, the free CP has latent enzyme activity [11]. Opening of the α ring requires the RP, which consists of two subcomplexes, the base and the lid. The RP base contains six different ATPase subunits (Rpt1 to Rpt6; RP triphosphatase) and four different non-ATPase subunits (Rpn1, Rpn2, Rpn10 and Rpn13: RP non-triphosphatase). The six-membered ATPase ring of the RP base, which is adjacent to the CP α ring, is responsible for the ATP hydrolysis that promotes unfolding of the substrate and its translocation into the CP cavity. Rpn10, a more distant RP base subunit, recognizes the poly-ubiquitin chain that marks a protein for proteasomal degradation [3]. The lid contains at least nine non-ATPase subunits. Rpn11, conferring an isopeptidase activity to the RP lid, cleaves off the ubiquitin moieties from the poly-ubiquitin chain of the target protein prior to its degradation [12,13].

Several proteasome-interacting proteins add to the plasticity of proteasome configurations. A high molecular mass protein, named Blm10 in yeast and PA200 in mammals, is the newest member of conserved proteasomal activators [14–16]. The potential function of Blm10 in nuclear proteasome activation is reflected by the hypersensitivity of *blm10Δ* mutants against DNA damaging agents (Doherty et al. 2012). Under normal growth conditions Blm10-associated proteasomes constitute a minor fraction of proteasome configurations. They predominate in proteasomal mutants affecting CP maturation [14].

Despite compelling *in vitro* evidence that in the absence of RP, the CP is able to degrade partly unfolded and unstructured non-ubiquitylated proteins [17], it remains a subject of debate whether this also occurs *in vivo* [18]. Significant degradation of misfolded proteins by the CP alone seems unlikely in living cells given the abundance of chaperones and ubiquitin-conjugating machineries that guide potential protein substrates to their native conformations or select them for degradation [19].

3. Localization of the proteasome in diving cells

Although it is now clear that proteasomes are largely nuclear in dividing cells (Fig. 1, left panels) and have the ability to move between the cyto- and nucleoplasm to carry out protein degradation at the right place and at the right time, the issue of proteasome movement in and out of the nucleus was initially controversial.

In the following paragraph I will review the history of proteasome localization studies and will also address the experimental limitations leading to different conclusions about proteasome localizations. Early studies by Franke and colleagues, which were later confirmed by Micesz and colleagues, localized proteasomes primarily to the nuclei of *Xenopus laevis* oocytes and cultured mammalian cells [5,20,21]. Subsequent studies that relied on different antibodies and fixation methods to examine mammalian cells revealed that the majority of proteasomal subunits were present in the cytoplasm [22]. Examination of cells from high cell density cultures revealed less pronounced nuclear staining and increased cytoplasmic staining. These observations shifted research to focus predominantly on cytoplasmic protein breakdown [23] and led to the notion that efficient degradation of nuclear substrates, such as the tumor suppressor p53 and cyclin-dependent kinase inhibitors, required their export to the cytoplasm [24,25]. Other studies, however, showed that proteasomes change their intracellular localization in cultured mammalian cells depending on the cell cycle stage [23]. Proteasomes were observed to accumulate at the

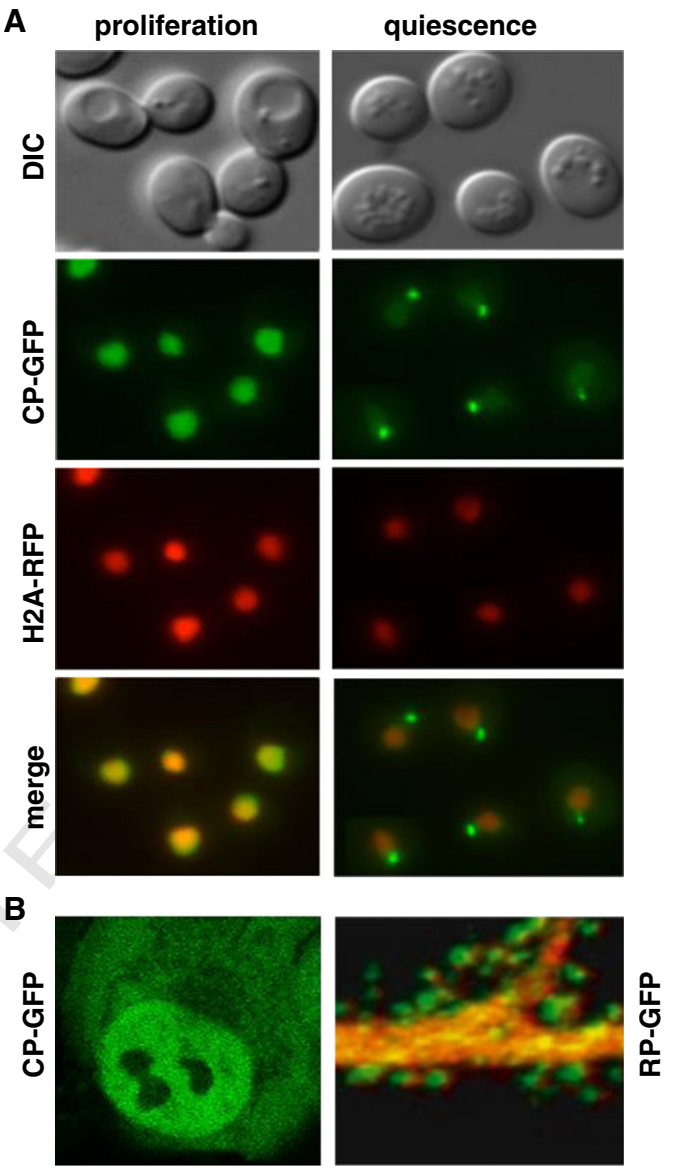

Fig. 1. *In vivo* localisation of GFP-labeled proteasomes during cell proliferation and quiescence. (A) Wild type yeast cells expressing GFP-labeled CP (via the reporter subunit β5) and cherry red (RFP)-labeled histone H2A were monitored by direct fluorescence microscopy using Nomarski optics (DIC), RFP and GFP filter sets. Dividing yeast cells are shown in the left panels, and quiescent yeast cells are shown in the right panels. Bar, 2 μm. (B) Micrograph of a human melanoma cell (line Mel JuSo) which was transfected with a GFP-tagged version of the CP subunit α3 (left panel). Live cell imaging of dendrites from a rat hippocampal neuron which was transfected with a GFP-tagged version of the yeast RP subunit Rpt1 [69]. Panel B was kindly provided by Florian Salomons and Nico Dantuma (Karolinska Institute). Image of dendrites was kindly provided by Erin Schumann (Caltech) with the license of the Nature Publishing Group.

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