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Roles of 26S proteasome in development and stress responses

Vai trò của 26S proteasome trong quá trình phát triển và đáp ứng stress

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Abstract

The 26S proteasome plays a central role in the proteolytic machinery. Organism relies on proteolysis to control key regulatory proteins and enzymes. Mutation of some subunits of the 26S proteasome affects growth and development. Additionally, these mutations altered stress responses such as heat, oxidative stress, etc. Recent studies showed that altered expression of some transcription factors resulted in abnormal gene expression of subunits of the 26S proteasome.

Keywords: Development; stress tolerance; 26S proteasome; gene expression; *Arabidopsis thaliana;* transcription factor.

Tóm tắt

26S proteasome đóng vai trò quan trọng nhất trong cỗ máy chuyển hóa protein của tế bào. Sinh vật dựa vào quá trình phân giải protein để kiểm soát các protein và enzyme chìa khóa. Đột biến các tiểu đơn vị của 26S proteasome làm ảnh hưởng đến quá trình sinh trưởng và phát triển của sinh vật. Bên cạnh đó, các đột biến này cũng làm thay đổi quá trình đáp ứng với stress của sinh vật như stress nhiệt, oxy hóa,... Một số nghiên cứu đã chỉ ra rằng 26S proteasome được kiểm soát bởi các nhân tố phiên mã. Biểu hiện bị biến đổi của các nhân tố phiên mã này làm thay đổi quá trình biểu hiện gene của các gene mã hóa cho 26S proteasome.

Từ khóa: Phát triển; đáp ứng stress; 26S proteasome; biểu hiện gene; Arabidopsis thaliana; nhân tố phiên mã.

1. Introduction

The 26S proteasome is the central proteolytic machinery of the highly conserved ubiquitin/proteasome system [18] [Fig. 1]. Organism relies on proteolysis to control key regulatory proteins and enzymes. The ubiquitin-26S proteasome system occupies nearly 6% of the *Arabidopsis thaliana* proteome [23]. Recently, the abundance of cell proteins regulating fundamental cellular processes such as proliferation, differentiation, cell cycling, hormone signaling, responses to

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environmental challenges or self recognition has been unraveled to undergo processing and functional limitation by entering the ubiquitin-proteasome degradation pathway with the final destination to be proteolytically degraded by the 26S proteasome [18, 23]. Protein degradation by the ubiquitin-proteasome system is necessary for a normal cell cycle [25]. In this work, we summarize some studies of roles of 26S proteasome in development and stress tolerance.

2. Altered expression of the 26S proteasome affects development

The loss of function of 26S proteasome/proteasomal subunits has been reported to induce abnormal phenotypes. RPN1 is implicated to play a crucial role during embryogenesis, a *rpn1a* mutant showed embryo lethality. Homozygous *rpn1a* mutant embryos are arrested at the globular stage with defects in the formation of the embryonic root, the protoderm, and procambium [4] had increased cell sizes [24]. Non-ATPase RPN5, a subunit of the regulatory particle, was shown to have a unique role in proteasomal function and *Arabidopsis* development. *rpn5a* mutants which caused abnormal embryogenesis, had a partially deetiolated development in the dark, and were severely dwarfed when grown in the light, and infertile [2]. Huang *et al.* (2006) identified *AE3* (*ASYMMETRIC LEAVES ENHANCER3*) encoding the putative 26S proteasomal subunit RPN8a. The *ae3 as2* double mutant shows severely abaxialized leaves. The double mutant not only enhanced expression levels of leaf abaxial promoting genes *FILAMENTOUS FLOWER, YABBY3, KANADI1* (*KAN1*) and *KAN2,* but also reduced expression level of the adaxial promoting gene *REVOLUTA (REV).* The authors hypothesized that the proteolytic function of the 26S holoenzyme was involved in leaf polarity formation [6]. *AE4* (*ASYMMETRIC LEAVES1/2 ENHANCER4*) encodes the RPN12a subunit of the 26S proteasome. *ae4* and *rev* mutants have minor defects in phyllotaxy and meristem initiation, whereas the *ae4 rev* double mutant displayed strong developmental defects. Expression of the *WUSCHEL* and *CLAVATA3* genes was severely decreased or absent in the late appearing *ae4 rev* primordia, but the genes were strongly upregulated in top-layer cells of inflorescence tips [26]. Kurepa *et al.* (2009) reported that optimal proteasomal function is required to maintain final shoot organ size in *Arabidopsis thaliana* [12]. Loss of function of RPT2a, a regulatory particle AAA ATPase subunit, caused a slight decrease in 26S proteasomal activity and led to enlargement of leaves, stems, flowers, fruits, seeds, and embryos. Enlarged leaves of *rpt2a* mutant caused by increased cell size resulted from extended endoreduplication early in leaf development [22]. Ueda *et al.* (2004) showed that *HLR* encoding the RPT2a subunit was expressed both in the RAM and in the SAM and that the activity of proteasome were reduced in the mutant. It is clear that loss of function of 26S proteasomal subunits alters plant phenotype, especially resulting in defects in primordial formation as well as RAM development. Partial loss of function of RPN10 and RPN12a, the regulatory particle non-ATPase subunits, resulted in an increase in cell size and a decrease in cell number [27]. Nguyen *et al*. (2012) observed an increase in leaf cell number in the null mutants of *RPX* with a downregulation of several 26S proteasomal subunit genes, whereas overexpression of *RPX* caused a dramatic decline in leaf cell number, along with upregulation of the 26S proteasome. Next to that, overexpressing the truncated version of *RPX* resulted in a severe reduction of root meristem cell number. Additionally, they also observed that deleting the C-terminus of

RPX causes early flowering, a reduction in rosette size, an induction of axillary branch formation, and in some cases the appearance of an aerial rosette and twisted leaves as compared to wild-type plants. Small siliques, clusters of small flowers (sometimes without petals) were also observed in oxΔc1 plants. Taken together, altered expression of the 26S proteasome or loss of function of proteasomal subunits result in severe defects of plant growth and development. Since in oxΔc1 plants the expression of the proteasomal genes is higher as in full-length overexpression plants, suggesting that the hydrophobic C-terminal domain might have a role in regulating protein stability [20]. In yeast, Rpn4, a TF that controls the expression of the proteasome, is itself also a target of ubiquitin-mediated protein degradation.

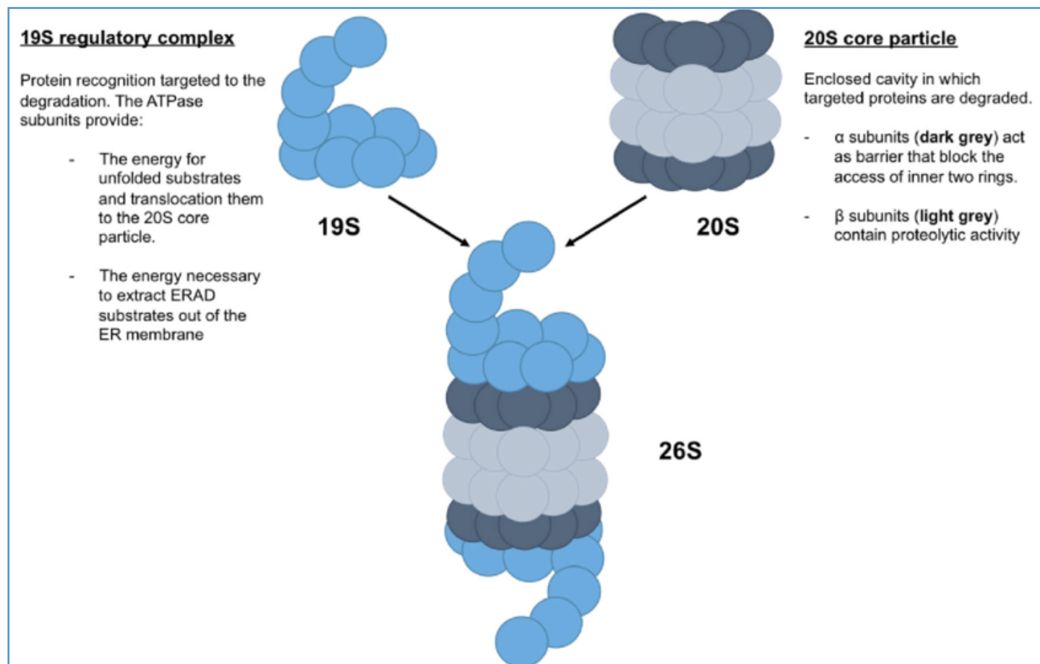

Fig. 1. Subunit composition of the 26S proteasome (schematic). [9]

3. Altered expression of the 26S proteasome affects stress tolerance

Recent studies implicated that altered expression of the 26S proteasome or proteasomal subunit mutants resulted in changes in stress responses. Wang *et al.* (2009) showed that *rpn1a* mutant plants decreased heat stress tolerance, increased oxidative stress tolerance [24]. Loss of function of RPT2a, RPN10 and RPN12a led to a decrease in 26S proteasomal accumulation but an increase in 20S proteasomal level. Seedlings carrying these mutants are hypersensitive to stresses [12]. Imai *et al.* (2003) showed a novel function of Hsp90 in the ATP-dependent assembly of the 26S proteasome. Loss of function of Hsp90 in yeast resulted in dissociation of the 26S proteasome. The authors also indicated genetic interactions between Hsp90 and several proteasomal *RPN* genes [7]. Repeated mild heat shock (RMHS) was reported to have several beneficial hormetic effects on human skin fibroblast undergoing aging *in vitro*. Beedholm *et al.* (2004) showed that serially passaged human skin fibroblasts exposed to RMHS at 41°C for 60 min twice a week increased 3 proteasomal activities by 40% to 95% in early- and midpassage cultures [1]. It is believed that an accumulation of unfolded proteins in cells is to be the common signal triggering the induction of heat shock proteins (HSPs). Accordingly,

Lee *et al.* (1998) treated *Saccharomyces cerevisiae* with the proteasomal inhibitor MG132 to inhibit protein breakdown at 30°C which caused a coordinate induction of many heat shock proteins within 1 to 2 h. Heat shock locked inactive 20S proteasome and impaired activation of the 26S proteasome. Proteasomal transcript levels were declined and the assembly of the proteasomal complex was inhibited after heat shock [15]. Heat shock also reorganized the cellular distribution of the proteasome [11]. Owsianik *et al.* (2002) showed that the *RPN4* promoter contains regulatory elements that bind Pdr1p and Pdr3p, two homologous zinc finger transcription factors. Mutations in the *RPN4* binding sites led to a decrease in expression of *RPT6.* Yap1p, a bZIP-type transcription factor, plays an important role in the oxidative stress response and multidrug resistance. A *cis*-element that binds Yap1p was identified in the *RPN4* promoter. These studies demonstrated that transcriptional regulators of multidrug resistance regulate the ubiquitin–proteasome system via *RPN4* expression [21]. Hahn *et al.* (2006) showed that in *Saccharomyces cerevisiae*, HSF activates expression of *PDR3,* encoding a multidrug resistance (MDR) transcription factor. MDR directly activates expression of *RPN4* transcription factor that directly regulates expression of a number of genes encoding proteasomal subunits. The HSF binding site (HSE) identified in the *RPN4* promoter is primarily responsible for heat- or methyl methanesulphonate induction of *RPN4.* Furthermore, heat-induced expression of *RPN4* led to expression of proteasomal genes at later stages of heat stress. HsfA2, heat shock transcription factor A2, is induced under environmental stress to defense against different stress types and regulates transcription of various defense-related genes [5]. Nishizawa-Yokoi *et al.* (2010) treated *Arabidopsis* T87 cells with MG132, or GDA (geldanamycin), a heat shock protein 90 (Hsp90) inhibitor. The results showed that transcript levels of *HsfA2* and its targets, *Hsp18.1-CI* and *ascorbate peroxidase 2* (*Apx2*), significantly increased [19]. In a previous study, *RPX* was isolated in a combination of high-light and heatshock stress-inducible genes [17]. In transcriptome studies, Nguyen *et al.* (2012) observed that some heat shock-responsive genes are downregulated. Overexpressing *RPX* showed a phenotype sensitive to heat and salt stress. The DNA binding site of HsfA2 (AGAACCTTCTAGAAT) was identified in the *RPX* promoter; however, direct binding of HsfA2 to the *RPX* promoter was not yet tested [20].

4. Upstream regulation of the 26S proteasome

In animal cells, several regulatory complexes interact with the 20S proteasomes and increase the amount of functionally distinct proteasome complexes. *γ*-Interferon upregulates three immuno beta-catalytic subunits of the 20S proteasome and the PA28 regulator and decreases the level of 26S proteasomes. Bose *et al.* (2004) showed that although phosphorylation of C8, a subunit of the 20S proteasome, was not completely essential for the formation of the 26S proteasome, it was subsequently needed to increase stability of the 26S proteasome [3]. Using a genome-wide scan, James *et al.* (2006) obtained evidence for potential modulation of proteasomal subunits and regulatory genes by the transcription factor Zif268 in neurons. Bioinformatic analysis indicated an enrichment of putative Zif268 binding sites immediately upstream of the transcriptional start sites of proteasomal genes. Transcript level regulation of genes encoding the Psmb9 (Lmp2) and Psme2 (PA28β) proteasomal subunits was confirmed after transfection of a neuronal cell line with Zif268.

Loss of function of Nrf1, a nuclear factor E2-related factor 1 (Nrf1) transcription factor, led to impaired proteasomal function. Gene expression profiling revealed a coordinate down-regulation of various proteasomal genes including PsmB6 (encoding a catalytic subunit of the proteasome) [8]. Transcriptional analysis and ChIP experiments demonstrated that PsmB6 is an Nrf1 target gene [14].

By using semiquantitative RT-PCR, Karpov *et al.* (2008) showed that deletion of *S. cerevisiae RPN4* decreased the *RAD6*, *RAD23* and *CDC48* mRNA levels, while the *UBI4* mRNA level increased. Rpn4p-dependent transcriptional upregulation of *RPT4* and *RPN5* were induced after heat shock stress. Apparently, Rpn4p acts both as an activator and a repressor of transcription of the ubiquitin - proteasomal genes under normal and stress conditions [10]. Mannhaupt *et al.* (1999) identified a new, unique upstream activating sequence (5P-GGTGGCAAA-3P) in the promoters of 26 out of the 32 proteasomal yeast genes characterized to date. By using the one-hybrid method, they showed that the factor binding to the proteasome-associated control element is RPN4p and confirmed by EMSA [16].

Growth of an organism and its size determination require the tight regulation of cell proliferation and cell growth. For leaf growth this involves the progression of component cells through a succession of developmental phases: proliferation (cells divide at a rate matching their expansion and maintain cell size homeostasis), expansion (cells stop dividing but continue to expand, typically to a size much larger than that of meristematic cells), and maturity (cells no longer expand). The size-control system is largely dependent on both cell size and cell number. However, final organ size is not simply the sum of cell number and cell size in plants, as evidenced by the abnormal enlargement of leaf cells that appears to be triggered by an insufficient supply of cells. In both the animal and plant kingdoms, body size is a fundamental but still poorly understood attribute of biological systems. The primary size of plant organs is influenced by environmental cues but mainly coordinated by endogenous signals that control cell proliferation and cell expansion. Nguyen *et at.* (2012) reported that RPX, a NAC transcription factor, affects cell proliferation during leaf development. Loss of function of RPX causes enlargement of leaves, whereas overexpression decreases leaf area by regulating the number of cells. Estradiol-inducible expression of *RPX* together with whole genome transcriptomics revealed that *RPX* activates the expression of genes encoding subunits of the 26S proteasome. Induction of *RPX* resulted in an increase in proteasomal activity and an increased abundance of proteasomal subunits that was confirmed by qRT-PCR with measurement of transcripts of all 26S proteasomal genes, transactivation assays, western blots and chemical labeling. Furthermore, they identified a conserved binding site for RPX in the upstream regulatory sequences (PRCE) of the proteasomal genes that was verified by IMAP, transactivation assays and ChIP-qPCR. With the achieved results, the authors concluded that RPX regulates expression of the genes encoding 26S proteasomal subunits and cell proliferation. In addition, the authors found that overexpressing RPX decreased stress tolerance (heat and salt stresses). Based on an analysis of promoter deletions, the authors performed an analysis of a 200-bp long region of the promoter using the web tool athamap. The analysis showed the presence of putative *cis*-elements including a binding site for heat shock factors (HSF), a cold response element (CBF), a binding site for a development-related factor (HVH21) that is recognized by knotted class 1 homeodomain protein, an abscisic acid-related

element (ABI4), and a salt-inducible element (ALFIN1). The functions of these elements can be studied in detail in the future. To better understand the integration of RPX into regulatory networks and to more precisely determine the mechanisms through which RPX controls development and stress tolerance it will also be important to identify upstream control elements [20].

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